





# ANNUAL REPORT VETERINARY MEDICINE 2009

Veterinary Medicine



Federal Ministry of Health Österreichische Agentur für Gesundheit und Ernährungssicherheit (Austrian Agency for Health and Food Safety) GmbH





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This 2009 report on veterinary medicine and consumer health shows that we can be very proud of our efforts and the animal health situation in Austria. We have all had a very busy year. Of particular note are the successes we have had in combating bluetongue disease. In the winter of 2008/2009, mandatory vaccination was introduced in Austria for all susceptible livestock in order to prevent the disease spreading rapidly across Europe.

Thanks to this initiative, instigated by federal and provincial veterinary authorities, agricultural stakeholders and breeding associations, we were able to achieve a vaccination rate in excess of 80 % across Austria. The success of this unique campaign is reflected in the fact that despite those cases detected in Austria in 2008, there was no indication of the circulation of this virus in 2009.

On a broad scale, this 2009 annual report provides the public with a good insight into the efforts that have been undertaken to ensure food safety, animal health and animal welfare in Austria.

In this context I would like to express my gratitude to all those who have worked together so well to deliver these positive outcomes in the area of animal health.

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# INTRODUCTION

The basic requirement for providing high-quality food products is to ensure the health of the animals producing them, achieved by accurate monitoring and control programmes. Animal disease monitoring and control in Austria is implemented in accordance with EU law, recommendations issued by the International Office of Epizootic Diseases and national legislation.

Successful implementation requires federal/provincial cooperation and the support of the veterinary medicine research facilities run by the Austrian Agency for Health and Food Safety ("AGES"). Monitoring programmes employing statistically verified sampling methods ensure that the health status of animals is tested annually across the country.



## OVERVIEW OF ANIMAL DISEASE SITUATION IN AUSTRIA

In 2009 Austria was free of the following highly contagious animal diseases:

Foot and mouth disease Rift Valley fever Vesicular stomatitis Sheep and goat pox Swine vesicular disease African swine fever Rinderpest (cattle plague) Classical swine fever Peste des petits ruminants Newcastle Disease Contagious bovine pleuropneumonia Avian influenza Lumpy skin disease African horse sickness



## **BLUETONGUE DISEASE**

Bluetongue disease (BT) was first diagnosed in South Africa in 1934. For a long time the disease was considered to be a tropical animal disease as it was only found around the world between the latitudes of 40° N and 35° S. Bluetongue disease is caused by a double-stranded RNA virus, a member of the genus *Orbivirus* and family *Reoviridae*, with 24 serotypes having being identified worldwide. The "Implementation of bluetongue monitoring in Austria" project was initiated in early 2007. This project is a joint project involving the Federal Ministry of Health - Veterinary Services, AGES and the Vienna Natural History Museum (NHM). In 2009 AGES investigated the presence of bluetongue antibodies (BTV-AB) and the presence of viral RNA (BTV-PCR) using real-time RT-PCR (Table 1). The Natural History Museum then recorded and counted the midge population caught in special traps (black blue light traps). Samples were taken from 54 locations in Austria in project year 2009 (Fig. 1).



Fig. 1: Location of vector traps in Austria



The samples numbered 6,932,731 individuals in total, with 51.5 % being identified as belonging to the genus *Culicoides*. The *Obsoletus* complex dominated with 92 % (3,284,728 individuals), followed by the *Pulicaris* complex (4.2 %, 149,955 individuals) and the *Nubeculosus* complex (0.7 %, 24,992 individu-

als). Other regular species were *C. furcillatus* (0.8 %), *C. fascipennis* (0.06 %), *C. circumscriptus* (0.03 %), and *C. duddingstoni* (0.03 %). 2.1 % of the samples could not be classified during routine examination (Fig. 2).



Fig. 2: Proportion of *Culicoides* complexes in Austria 2009



The BTV diagnosis for ruminants and camelids involved veterinarians taking blood or milk samples from live animals and organ samples from dead animals. These were tested in the AGES institutes of veterinary medicine in Mödling (milk, blood and organ samples), Linz and Innsbruck (blood samples) for BTV-AB and BTV-PCR. A total of 60,540 tests for bluetongue disease were conducted in 2009 (Table 1).

|                                |           | Tierart         |           |            |          |          |            |                 |  |
|--------------------------------|-----------|-----------------|-----------|------------|----------|----------|------------|-----------------|--|
|                                | US        | Rind            | Schaf     | Ziege      | Wildwdk  | Kamelide | Culicoides | Σ               |  |
| BT Monitoring<br>1266          | PCR       | 6.921           | 123       | 6          |          |          |            | 7.050           |  |
| BT Monitoring<br>UND Import-US | PCR       | 111             | 120       |            |          |          |            | 231             |  |
| Sentinel                       | AK<br>PCR | 27.676<br>2.620 |           |            |          |          |            | 27.676<br>2.620 |  |
| Import                         | AK<br>PCR | 120<br>584      | 72<br>635 | 16<br>30   | 2<br>14  | 8<br>7   |            | 218<br>1.270    |  |
| Routine                        | AK<br>PCR | 7.020<br>13.522 | 72<br>224 | 204<br>266 | 119<br>4 | 5<br>5   | <br>34     | 7.420<br>14.055 |  |
|                                | Summe     | 58.574          | 1.246     | 522        | 139      | 25       | 34         | 60.540          |  |

Table 1: Number of BT tests for ruminants, camelids and culicoides\* on the basis of the AB and PCR tests

\*) RT-PCR tests on trapped, blood filled culicoides commenced in November 2009.

Austria has been a uniform zone since December 15, 2008. Samples were gathered from ruminants in the two BTV programmes, "Sentinel" and "BT monitoring 1266", in the 28 Sentinel regions. "BT monitoring 1266" is a virological study using Real-time RT-PCR to compensate for deficits in the number of sentinel animal examinations. In the "Sentinel programme" the blood or milk of cattle is examined for antibodies, while in the "BT monitoring 1266" examines blood samples from cattle and small ruminants are examined for viral genome.

From December 15, 2008 to March 31, 2009 all cattle, sheep and goats in Austria had to be mandatorily vaccinated against BTV pursuant to Section 7 of the Bluetongue Control Regulation (BGBI. II Nr. 267/2008). Since 31/03/2009 livestock owners have been able to voluntarily vaccinate susceptible animals at their own expense. The Federal Ministry of Health expressly recommends conducting vaccination and therefore maintaining vaccination protection.

In 2009, 17 BTV cases of BT were found during the course of blood serum monitoring tests in cattle. Of these, 14 BTV cases were in the federal province of Upper Austria, two BTV cases in the province of Vorarlberg and one BTV case in the province of Salzburg (Table 2). Considering when these cases were discovered, it can be assumed that the animals were infected with the disease in autumn 2008 and therefore no virus circulation occured in Austria in 2009.

Table 2: Positive BTV cases in Austria in 2009

| Anzahl | Bundesland     | Bezirk    | Tierart |
|--------|----------------|-----------|---------|
| 14     | Oberösterreich | Schärding | Rind    |
| 1      | Salzburg       | Hallein   | Rind    |
| 1      | Vorarlberg     | Bregenz   | Rind    |
| 1      | Vorarlberg     | Bludenz   | Rind    |

### BOVINE BRUCELLOSIS, ENZOOTIC BOVINE LEUKOSIS AND IBR/IPV

For the last two years bulk tank milk from holdings supplying milk have been monitored by testing for brucellosis, enzootic bovine leukosis (EBL) and infectious bovine rhinotracheitis / Infectious pustular vulvovaginitis (IBR/IPV). In 2009 a total of 35,973 samples of tank milk were tested using ELISA at the AGES Institute of Veterinary Medicine in Linz, with 183 samples (0.5 % of the businesses studied) having a non-negative outcome. Non-negative bulk tank milk examination results led to 4,045 blood samples being taken from the affected herds for further investigation at the AGES Institute of Veterinary Medicine in Mödling (Table 3).

#### Table 3: Results of the tank milk tests

|            | Tankmilch-<br>Untersuchungen   |  |  |  |  |  |
|------------|--------------------------------|--|--|--|--|--|
|            | Gesamt Nicht-negative Betriebe |  |  |  |  |  |
| Brucellose | 35.973 94 (0,26 %)             |  |  |  |  |  |
| ERL        | 35.973 24 (0,07 %)             |  |  |  |  |  |
| IBR/IPV    | 35.973 65 (0,18 %)             |  |  |  |  |  |

In order to monitor non-milk supplying holdings again this year, 27,366 blood samples were taken as part of a risk-based sampling plan and tested at the AGES Institute of Veterinary Medicine in Mödling for brucellosis, EBL and IBR/IPV. In 2009 no cases of enzootic bovine leukosis or brucellosis were established on any holding. IBR/IPV was diagnosed in one herd and two herds were found to contain seroreagents with antibodies from vaccination.



# TUBERCULOSIS

Human and animal tuberculosis (TB) is caused by acid-fast mycobacteria, included in the so-called *M. tuberculosis* complex. The complex encompasses *M. tuberculosis*, *M. africanum*, *M. canetti*, *M. bovis*, *M. caprae*, *M. pinnipedii* and *M. microti*. Today, the bacteria are differentiated and classified mainly by molecular biological methods based on specific gene sequences, including polymerase chain reaction, restriction fragment length polymorphism and spoligotyping.

In Austria, bovine tuberculosis is a notifiable animal disease. Pursuant to a Commission Decision, Austria has been recognised as being officially free of bovine tuberculosis (*M. bovis*) since 1999. Since then there has been no intradermal testing of ruminants and the disease is now monitored as part of ante-mortem and post-mortem inspections.

In 2008, an open form of pulmonary tuberculosis (infectious agent: *M. caprae*) was detected in a slaughtered bovine from Lechtal in the Tyrolean district of Reutte during the course of ante-mortem inspections. Subsequently, the disease was diagnosed in numerous animals in the herd of origin as well as in cattle from contact holdings. As there is a tradition of holding many young cattle from various Tyrolean districts in Lechtal's alpine pastures, in autumn 2008 the Federal Ministry of Health ordered all cattle requiring testing from the age of six weeks to be tuberculin tested in the districts of Reutte, Landeck, Imst and Innsbruck-Land.

As a result of this prescribed tuberculin testing in 2009, *M. caprae* was bacteriologically confirmed in 4 diagnostically slaughtered animals, all from one cattle holding in the district of Innsbruck-Land. Further investigations tend to indicate that the disease was introduced into the holding when a cow was purchased from external sources. It had already died from a chronic lung condition at the time of the tuberculin test. One bovine from Reutte district was also found to be *M. Caprae* positive in 2009.

All isolates of the TB positive cattle were identified as the TB pathogen *M. caprae*. The DNA fingerprints collected so far match the isolates from the Landeck and Reutte districts with those found in recent years in cattle and wild red deer from Lechtal in Tyrol, as well as with individual red deer from the district of Bludenz in Vorarlberg. This DNA fingerprint has also been found in isolates of TB positive cattle in the neighbouring Allgäu region in Bavaria, Germany.

Five positive pathogenic *M. caprae* isolates from two holdings were reported in Tyrol in 2009.

## BOVINE VIRAL DIARRHOEA / MUCOSAL DISEASE

The disease is found globally and is caused by a pestivirus belonging to the *Flaviviridae* family. Cattle infected persistently with the BVD virus excrete it throughout their entire lives and are responsible for spreading the disease. BVD/MD is one of the most economically significant infectious diseases in cattle. Consequently, other countries have also opted to combat the disease actively at a national level (Switzerland, Sweden).

The majority of the complex of symptoms often occur unnoticed. Respiratory tract infections, diarrhoea, fever, loss of appetite, reduced milk production and a general weakening of the immune system are possible. In most cases animals experience fertility problems, resulting in abortions or the birth of deformed and weak calves. Infection with the BVD virus triggers a transitory infection that usually goes undetected. Subsequently, this acute or transient infection results in the development of antibodies which can be detected in the blood or milk. A special form of BVD in persistently infected animals is mucosal disease. The disease is particularly severe, resulting in the death of the infected animals. Typical symptoms are massive and often bloody diarrhoea, high fever, extreme mucosal erosions and subsequent secondary infections. BVD control in Austria has been regulated by the BVD Regulation since August 2004. Diagnosis is made on the basis of the detection of antibodies in blood, individual milk or bulk tank milk samples. Blood, tissue, secretion and organ samples are suitable for ascertaining the presence of the virus (antigen detection).

The distribution of BVD virus-free, BVD suspected and BVD infected holdings in the individual federal provinces are listed in Table 4.

|                  | BVD-virusfreie<br>Bestände <sup>1</sup> | BVD-verdächtige<br>Bestände <sup>2</sup> | BVD-infizierte<br>Bestände <sup>3</sup> |
|------------------|---|--|---|
| Burgenland       | 443                                     | 3  | 1                                       |
| Kärnten          | 8.042                                   | 90                                       | 18                                      |
| Niederösterreich | 10.951                                  | 3  | 10                                      |
| Oberösterreich   | 9.147 (13.336*)                         | 348                                      | 32                                      |
| Salzburg         | 6.380                                   | 1  | 17                                      |
| Steiermark       | 12.927                                  | 491                                      | 3                                       |
| Tirol            | 9.512                                   | 239                                      | 25                                      |
| Vorarlberg       | 2.343                                   | 226                                      | 27                                      |
| Wien             | 1                                       | 0  | 0                                       |

Table 4: No. of BVD virus-free, BVD suspected and BVD infected cattle holdings

<sup>1</sup> Virus-free holding: Officially recognised BVD virus-free holding

<sup>2</sup> Suspected holding: A holding where bulk tank milk, milk and blood tests or clinical symptoms indicate the presence of the pathogen

<sup>3</sup> Infected holding: A holding with at least one proven persistently infected animal (PI)

\* Officially recognised BVD virus-free holdings and holdings with an unsuspicious survey test result in 2009 (reason for holdings not being officially recognised as BVD virus-free is usually the protracted interval between tests)

# PARATUBERCULOSIS

Paratuberculosis or Johne's Disease is a chronic and incurable intestinal infection in ruminants. This global disease was first described by Johne and Frothingham in 1895. Paratuberculosis is caused by Mycobacterium avium subspecies paratuberculosis (MAP). The infection is usually transmitted to young animals from faeces, milk contaminated with faeces, teats and colostrum. Following an incubation period of 2 - 10 years, the disease is characterised by uncontrollable diarrhoea despite the maintenance of appetite, emaciation, lower milk production and reduced weight gain, infertility and death. Clinical paratuberculosis in cattle, sheep, goats and wild ruminants in game holdings has been a notifiable disease in Austria since 03/04/2006. Testing within the scope of this monitoring programme regulated under a regulation to combat clinical paratuberculosis is conducted centrally at the AGES Institute of Veterinary Medicine in Linz (IVET LINZ). The aim is to detect animals suffering from clinical paratuberculosis and remove them from holdings.

Clinically suspicious cases can be investigated diagnostically by submitting blood and faecal samples to the testing authority. Organ material (intestinal samples, lymph nodes) is submitted for animals that have died or have been killed. In 2009, samples from 109 cattle (64 holdings), 2 sheep (2 holdings) and one wild ruminant in captivity (1 holding) were examined within the scope of the monitoring programme. An MAP infection was detected in 39 cattle. The positive cattle came from 24 holdings, with limousin (18 animals) and Fleckvieh (7 animals) being the most frequently represented breeds. Figure 4 shows the clinically suspicious cases for the individual federal provinces (numbers in black), the number of MAP-positive animals (numbers in red) and the number of MAP positive holdings (numbers in blue).



**Fig. 4:** Number of suspicious cases (black), animals confirmed by positive laboratory results (red) and the positive holdings (blue) for the individual provinces.



## TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSE) - BSE, SCRAPIE, CWD

The 2009 TSE monitoring programme fulfils the legal provisions of Regulation (EC) No. 999/2001, the Scrapie Surveillance Regulation and the current version of the TSE Notification (AT). In addition, processing of bar-coded submissions was tested in cooperation with the Federal Consumer Information System ("VIS") in 2009 (see Figure 5).

#### Cattle

The applicable age limit is subject to the animal's country of birth as provided for in Section 2 of the TSE Notification (also see Table 5).

The following animals were tested for BSE: All cattle from 30 and 48 months of age (depending on country of birth) slaughtered for human consumption

- all cattle that died or were killed from 24 months of age
- all sick or emergency slaughtered cattle from 24 or 48 months of age

All these tests were concluded with a negative outcome.

There were no cases of BSE in Austria in 2009. There have been a total of 6 cases of BSE in Austria prior to the end of 2009.



**Fig. 5:** BSE sample container for submission with VIS barcode labelling



|                             | getestet ab<br>einem Alter von | AGES<br>Innsbruck | AGES<br>Linz | AGES<br>Mödling | (LA<br>Klagenfurt) | Gesamt<br>Österreich |
|-----------------------------|--------------------------------|-------------------|--------------|-----------------|--------------------|----------------------|
| Schlachtrinder              |                                |                   |              |                 |                    |                      |
| aus Österreich (A)          | 48 Mon                         | 15.663            | 42.837       | 58.927          | 10.030             | 127.457              |
| Schlachtrinder aus          |                                |                   |              |                 |                    |                      |
| D, F, B, GB, FI, DK, I, IR, |                                |                   |              |                 |                    |                      |
| GR, LU, NL, P, SW, SP       | 48 Mon                         | 77                | 39           | 1.437           | 701                | 2.254                |
| Schlachtrinder              |                                |                   |              |                 |                    |                      |
| aus allen anderen           |                                |                   |              |                 |                    |                      |
| Ländern                     | 30 Mon                         | 2.452             | 10.641       | 24.382          | 3.315              | 40.790               |
| Auf Ersuchen                |                                |                   |              |                 |                    |                      |
| Verfügungsberechtigter      | 20 Mon                         | 0                 | 2            | 0               | 0                  | 2                    |
| Not- und Sonder-            | 24 bzw.                        |                   |              |                 |                    |                      |
| schlachtungen               | 48 Mon                         | 113               | 834          | 727             | 81                 | 1.755                |
| Verendete und               |                                |                   |              |                 |                    |                      |
| getötete Rinder             | 24 Mon                         | 4.040             | 4.673        | 7.898           | 2.634              | 19.245               |
| Klinische                   |                                |                   |              |                 |                    |                      |
| Verdachtsfälle              |                                |                   |              | 2               |                    | 2                    |
| Gesamt pro Institut         |                                | 22.345            | 59.026       | 93.373          | 16.761             | 191.505              |

**Table 5:** The exact test numbers on the basis of the statutory test categoriesand the institutes conducting them.



### **Sheep and goats**

In April 2006 Austria was granted additional guarantees for scrapie from the European Union. A national monitoring programme ensures that an outstanding disease situation is maintained. The following animals are tested within the scope of this monitoring system (see Table 6):

- all sheep and goats over 18 months of age that have died or been killed and
- slaughtered sheep and goats from 18 months of age,

a) when animals were introduced into Austrian holdings from countries where scrapie is endemic or where scrapie has been confirmed within three years prior to introduction of the animals, or

b) when scrapie is confirmed in their holding of origin within three years of the purchased animals being introduced.

No case of scrapie was detected in 2009.

Table 6: The exact test numbers on the basis of the statutory test categories.

| Untersuchungskategorien  | Anzahl |
|--|--------|
| Geschlachtete Schafe und Ziegen über 18 Monate aus gefährdeten Betrieben | 16     |
| Verendete Schafe   | 5.906  |
| Verendete Ziegen   | 1.808  |
| Klinische Scrapie Verdachtsfälle   | 1      |
| Summe  | 7.731  |



### Cervids

The time-restricted deer testing programme launched in the EU in 2007 concluded in March 2009.

There was no evidence of CWD in Austria in the reporting year.





# CAMPYLOBACTERIOSIS

Enzootic campylobacter abortions in cattle constitute a notifiable specific venereal disease clinically characterised by a large number of fertility disorders and abortions. The infection is found worldwide, but now occurs more sporadically following the introduction of artificial insemination. The infectious agent is *campylobacter fetus subspecies venerealis*, a gram-negative S-shaped spirally curved bacterium. This pathogen is preferentially detected from foetal abomasia or preputial washings in culture or using PCR.

Of the 2,081 samples submitted in 2009, two delivered a positive result.





## BRUCELLOSIS IN SMALL RUMINANTS

### Brucella melitensis

The zoonosis known as Malta fever in human cases is caused by the bacterium *Brucella melitensis (B. melitensis)*. In sheep and goats it leads to problems with the reproductive organs and in rare cases also to inflammation of the joints and massive discharges of pathogens in amniotic fluids, foetal membranes, aborted foetuses, vaginal secretions and milk. The primary source of infection for humans is eating cheese made from raw sheep's or goat's milk. *B. melitensis* infections are endemic to the Mediterranean region and were also detected in several Balkan countries in 2009.

Pursuant to Commission Decision No. 2001/292/ EC, Austria has been declared officially free of *B. melitensis* since April 11, 2001. This status is to be confirmed by means of annual representative sample tests. The sample size will be published in the Official Veterinary Bulletin by the competent federal ministry. A negative test result must be obtained for international trade in animals.

Blood samples from 9,552 sheep and 2,938 goats were tested for *B. melitensis* antibodies in 2009.

One case of B. melitensis was officially detected in small ruminants in 2009.

### Brucella ovis

3,285 tests were conducted in 2009. Antibodies to the Infectious Epididymitis pathogen were detected in a total of five rams in the course of serological testing.



## RABIES

In 2009 Austria was also able to maintain its rabies free status that was officially declared on September 28, 2008.

In 2009, a total of 8,826 animals were tested for rabies using direct immunofluorescence in the National Reference Laboratory (NRL) for Rabies at the Institute of Veterinary Medicine in Mödling (IVET Mödling). Of these, 8,674 samples came from wild animals, primarily foxes, and 152 samples from pets. The number of bats tested rose from 68 in 2008 to 360. In addition, pursuant to the provisions of the Manual for Diagnostic Tests and Vaccines for Terrestrial Animals 2009, virus isolation in cell culture was performed for 158 animals that had either bitten humans or shown suspicious behaviour while having had contact with humans. In addition, cerebral and salivary gland immunohistochemical testing was also undertaken (Fig. 7). If the material does not allow virus isolation, molecular biological methods are used (2009: 10 cases). Molecular biological methods and immunohistochemistry are the most important methods used at the NRL for ante-mortem diagnosis in suspected human cases. The preferred test methods are nuchal skin biopsies and pharyngeal, conjunctival and nasal swabs. In 2009 no human samples were sent for testing.

Positive isolates are typed by sequencing in the Molecular Biology Unit to obtain further valuable epidemiological data. As was the case last year, the accrued animal material is used for further scientific examination. 46 cats were tested immunohistochemically for feline spongiform encephalopathy (FSE), all producing a negative result. Research into the spread of fox tapeworm (Echinococcus) in the federal province of Styria is in its final stages. The investigations (detection of *Brucella sp.* and *Franciscella tularensis* in fox lymph nodes) delivered interesting results and are continuing.







**Fig. 6:** Number of confirmed rabies cases (1985 – 2009)

Although Austria was declared rabies free on September 28, 2008, due to current cases of rabies in Slovenia and Italy vaccination will continue twice a year in a vaccination belt in southern Austria (southern areas of Burgenland, Styria and Carinthia) by way of aerial drops of rabies baits. Despite several rabies bait drops in Italy, rabies has spread westward, so that at the end of 2009 the Belluno region was also affected, in addition to the Udine and Pordenone regions. Therefore, an emergency distribution of rabies baits in the southern part of East Tyrol and western regions of Carinthia was undertaken in Austria at the end of 2009 (Fig. 8).

As part of the 2009 spring vaccination campaign, 296,800 vaccination baits containing an attenuated live vaccine were dropped by air over an area of 10,685 km<sup>2</sup> (North East Lower Austria, all of Burgenland, southern parts of Styria).





The vaccination programmes and the favourable disease situation in the Czech Republic, Slovakia Republic and Hungary meant that in autumn 2009 Lower Austria and northern Burgenland had their vaccination area status reversed.

Consequently, the number of baits dropped in autumn was reduced to 120,000 over 4,790 km<sup>2</sup> in the southern border region of Styria, Carinthia and Burgenland. 21,600 baits were used for the emergency vaccination campaign over 854 km<sup>2</sup> in parts of Carinthia and East Tyrol in December 2009. Virus titre tests for three vaccine batches were performed at the NRL in 2009. All fulfilled the requirements.



**Fig. 7:** Detection of rabies antigen in a fox brain using immunohistochemistry



**Fig. 8:** Vaccination area for East Tyrol / Carinthia emergency vaccination, December 2009

The success of vaccination is determined on the one hand by detecting the tetracycline marker added to the vaccination bait in the fox's jawbone using UV autofluorescence (uptake rate of vaccination bait) and on the other hand by detecting antibodies in the blood using a commercially available ELISA (vaccination efficacy). In the 2008/2009 season (submission of samples started six weeks after autumn vaccination, i. e. mid to late December), 464 of the 525 jawbones tested from the vaccination area were positive, giving an uptake rate of 88.38 %. 35.14 % of the 481 blood samples tested returned a positive result. With these results Austria is on par with the other European countries which use this test and like Austria - implement a successful rabies control programme. At the Rabies Serology Meeting held in Nancy in 2009 following the Second Workshop for Rabies a new more sensitive ELISA for testing fox blood samples was presented. The NRL was represented at the events by two of its staff. There are plans to put this test to trial and to establish it after validation if the evaluation is positive.

In 2009 the NRL participated successfully in two interlaboratory rabies tests, , allowing it to demonstrate its own level of expertise.

Within the scope of testing for the PET Travel Schemes and Human Vaccination Success Monitoring, 951 blood samples were tested using the Fluorescence Antibody Virus Neutralisation Test (FAVNT) in 2009. 853 samples were from dogs, 89 from cats and 9 from humans. Of these, 804 samples displayed a sufficiently high antibody titre, 126 an insufficient titre and 21 samples could not be evaluated.





## CLASSICAL SWINE FEVER (CSF)

1,486 swine blood samples were tested for CSF antibodies in the National Reference Laboratory at IVET Mödling. Of these, 1,342 tests were commissioned privately and 144 officially. 124 organ samples were tested in cell culture and 12 samples in RT-PCR for evidence of CSF, with six commissioned privately.

Neither antibodies nor virus were detected in any of the samples.

Table 7: No. of samples tested for CSF 2009

| Antikörper<br>(ELISA) | Virus-<br>isolierung | RT-PCR |
|-----------------------|----------------------|--------|
| 1.486                 | 124                  | 12     |

## AUJESZKY'S DISEASE (AD)

In 2009, 12,560 blood samples from domestic pigs were tested for AD antibodies.

76 organ samples were tested for evidence of the virus in cell culture and six samples using RT-PCR.

All tests returned a negative result.

Table 8: No. of samples tested for AD in 2009

| Antikörper | Antikörper | Antigen           | PCR |
|------------|------------|-------------------|-----|
| (ELISA)    | (SNT)      | (Virusisolierung) |     |
| 12.560     | 11         | 76                | 6   |

## ZOONOSIS BASELINE STUDY: SALMONELLA AND CAMPYLOBACTER

Within the scope of zoonosis and zoonosis pathogen monitoring in accordance with the Austrian Zoonosis Act, abattoir batches of fattening hens were tested for thermotolerant campylobacter and cattle and sheep for verotoxigenic *Escherichia coli* (VTEC).

The results of the tests are shown in Table 9. 45 % of the samples tested positive for *Campylobacter jejuni* or *C. coli*. The figures lie slightly below the results of the 2008 "Campylobacter" baseline study, with a total of 52 % positive samples.

The VTEC tests show that many more samples were positive using ELISA than could be confirmed using PCR. This can also be attributed to the fact that only some of the *E. coli* colonies found in the culture could be tested using PCR. It was also demonstrated that fleece does not constitute a suitable alternative material for sheep as considerably more positive results could be achieved using swaps (see Table 10 and 11). If the PCR results are applied to the total samples tested, 18 % of calves, 24 % of cattle and 46 % of sheep tested positive for VTEC. In comparison, the detection rates in 2008 were 16 % for calves, 15 % to cattle and 21 % in sheep.

Table 9: Results of the campylobacter tests for poultry

| C. coli und C. jejuni     | 147 | 45 %  |
|---------------------------|-----|-------|
| C. coli                   | 52  | 16 %  |
| C. jejuni                 | 95  | 29 %  |
| neg.                      | 176 | 54 %  |
| nicht untersucht (zu alt) | 2   | 1 %   |
| Gesamt eingesandt         | 325 | 100 % |
| Vorgabe Stichprobenplan   | 312 |       |

Table 10: Results of the VTEC tests using ELISA

| Tierart           | Material | Ergebnis |                          |          |            |                 |
|-------------------|----------|----------|--------------------------|----------|------------|-----------------|
|                   |          | negativ  | positiv nicht untersucht |          | gesamt     |                 |
|                   |          |          |                          | (zu alt) | eingesandt | Stichprobenplan |
| Kalb (< 6 Monate) | Tupfer   | 50       | 44 (46%)                 | 6        | 100        | 96              |
| Rind              | Tupfer   | 34       | 44 (56%)                 | 6        | 84         | 80              |
| Schaf             | Vlies    | 56       | 28 (33%)                 | 17       | 101        | 90              |
|                   | Tupfer   | 19       | 65 (77%)                 | 17       | 101        | 90              |

**Table 11:** Results of the confirmatory tests using PCR for

 VTEC-ELISA positive isolates (see Table 2)

| Tierart           | Material | Ergebnis |         |        |
|-------------------|----------|----------|---------|--------|
|                   |          | negativ  | positiv | gesamt |
| Kalb (< 6 Monate) |          | 27       | 17      | 44     |
| Rind              |          | 25       | 19      | 44     |
| Schaf             | Vlies    | 25       | 3       | 28     |
|                   | Tupfer   | 27       | 38      | 65     |

# AVIAN INFLUENZA

In 2009, 4,147 blood samples were tested for AI antibodies: 3,977 samples with ELISA and 170 samples with HAH. 35 organ samples were tested for virus replication in embryo culture and 2,108 wild birds and 36 commercial poultry for the virus genome in real-time RT-PCR.

The pan-European AI screening programme consists of an active and a passive component. Serological testing was undertaken in active surveillance on the slaughter blood of 650 laying hens from 65 holdings, 820 fattening turkeys from 82 holdings, 1,970 geese and ducks from 71 holdings and 74 ostriches from seven holdings. Faecal swabs from 401 wild waterbirds and 321 swabs from sentinel ducks from the "Constanze" project were tested for evidence of the virus using real-time RT-PCR.

Passive surveillance involved testing 244 organs and 1,142 tracheal and cloacal swabs from birds found dead by means of real-time RT-PCR and 1,347 dissections on birds for AI.

#### All antibody tests returned negative results.

No HPAI could be detected by means of RT-PCR viral detection. 53 avian influenza viruses, including 5 cases H5 LPAI and 1 case H7 LPAI were found.

Table 12: No. of samples tested for AI in 2009

| Antikörper ELISA | Antikörper HAH | Realtime RT-PCR | Eikultur |
|------------------|----------------|-----------------|----------|
| 3.977            | 170            | 2.144           | 35       |



# NEWCASTLE DISEASE

Newcastle Disease (ND, atypical fowl pest) is a highly contagious acute to chronic avian disease. The virus belongs to the family of Paramyxoviruses, a singlestrand RNA virus. The disease is characterised by rhinitis symptoms, CNS symptoms and diarrhoea. It is associated with high morbidity and mortality, particularly amongst pigeons. A distinction is made between apathogenic, lentogenic (slightly pathogenic), mesogenic (slightly virulent) and velogenic (highly virulent) types of viruses. Vaccination is permitted in Austria and is performed for chickens, geese and pigeons (carrier and breeding pigeons). In individual cases these viruses can cause conjunctivitis in humans.

ND is a notifiable disease. The appearance of clinically suspicious symptoms must be reported to the official veterinarian, who will submit samples for diagnosis. Only highly pathogenic types of viruses are reported as an epidemic when the virus has a pathogenic index (ICPI) of 0.7 or above and when pathotyping of the virus strain from the amino acid sequence using sequencing of the fusion protein F0 fragment shows it to be velogenic. "Multi-basic amino acids" at the C-terminal end of the F2 protein and phenylalanin at amino acid position 117 - the N-end of the F1 protein - serve as criteria. The "multi-basic amino acids" refer in this case to 3 arginine or lysine terminals between position 113 and 116.

Different provisions apply for commercial poultry than for pigeons kept in captivity (carrier pigeons). The laboratory diagnosis is determined by detecting the pathogen from tracheal/oropharyngeal swabs and cloacal swabs as well as from animal bodies (CNS, lung, liver, spleen, gut) by replicating viruses in embryo culture and subsequent haemagglutination (HA) and haemagglutination inhibition tests (HAI) as well as molecular biological methods (RT-PCR and additional pathotyping). Detection of antibodies using ELISA and HAI is possible, but must be evaluated in context where vaccination is permitted.

The number of ND tests in 2009 is shown in Table 13.

### Table 13: ND tests

| Antikörper mittels HAH | Virusnachweis mittels Eikultur | Erregernachweis mittels RT-PCR |
|------------------------|--------------------------------|--------------------------------|
| 1.257                  | 28                             | 38                             |

Antibody testing is carried out for vaccination monitoring purposes mostly.

Positive identification of the virus in pigeons or wild doves occurred in 4 cases.

## PSITTACOSIS (ORNITHOSIS, PARROT DISEASE)

This disease is notifiable when detected in psittaciforms (parrots and parakeets). This disease is known as ornithosis with respect to other birds. Psittacosis is a zoonosis that occurs globally.

The pathogen is the gram-negative bacterium *Chlamydophila (Chl.) psittaci.* It appears in different embodiments such as elementary bodies, the infectious form, intermediary bodies and initial bodies and is inevitably intracellular. The individual species of Chlamydophila adapt very well to their host, *Chl. psittaci* to psittacidae, *Chl. abortus* to sheep/goats, *Chl. trachomatis* to humans.

Humans can also contract psittacosis. Infection is usually aerogen from aspirating infectious faeces and dust. The resulting symptoms are usually a general fever and subsequent pneumonia. The incubation period is 3 - 29 days, but up to 100 days has also been reported. Symptoms in birds include pneumonia, coughing, emaciation, ruffled feathers, diarrhoea, ophthalmic and nasal discharge. Death can occur from between a few days to several weeks or the disease becomes chronic, with the animals appearing to recover but continuing to discharge pathogenic agents.

The treatment of choice is tetracycline (oxytetracycline), which needs to be administered longterm. Prevention involves birds being quarantined and tested for Chlamydophila. Standard hygiene measures must be observed when working with the animals.

The number of tests conducted in 2009 is shown in Table 14.

#### Table 14: Tests for Chlamydophila sp.



*Chlamydophila psittaci* was detected in psittacidae in three cases.



# WEST NILE VIRUS

West Nile Virus (WNV), the infectious agent of West Nile fever, belongs to the *Flaviviridae* family and is included amongst the comprehensive group of arboviruses (arthropod-borne viruses). Arboviruses are viruses that can multiply in bloodsucking arthropods and be transmitted to vertebrates and humans. Amongst the flaviviruses, together with other meningitis and encephalitis inducing viruses, WNV belongs to the group of Japanese encephalitis viruses (JEV). It is transmitted to animals (primarily birds and horses) and humans by infected midges. The disease can not be transmitted either between horses or from horses to humans as not only humans but also horses constitute aberrant hosts for the virus, i. e. a dead end. It is assumed that the disease is transmitted by migrating birds during their migrations.

In 80 % of cases in humans the WNV lineage 2 infection is asymptomatic. In the event of symptoms, they are mild and do not provide a clear indication of a WNV infection. The endemic appearance of lineage 1 WNV in humans and horses in the north of the Italian province of Ferrera has been confirmed since 2008. No instance of WNV was diagnosed in animals in Austria until 2008. In 1989 a test of small mammals in Austria revealed the presence of WNV antibodies. As WNV has appeared in animals in Hungary since 2004, it was probably only a matter of time until the virus reached Austria and spread. In August 2008, the first clinical WNV infections with lineage 2 (less virulent for humans) in birds of prey (predominantly hawks, one falcon) were detected in Vienna and in eastern Lower Austria. A year later, the infection was detected again in a hawk in western Lower Austria. The infection was lethal for these birds of prey. Clinical cases in horses or humans have not yet occurred in Austria.



#### Table 15: WNV tests: Comparison 2008 – 2009

|                               | 2008              | 2009              |
|-------------------------------|-------------------|-------------------|
| Aviäre Influenza              |                   |                   |
| Überwachung (Organe)          | 17                | 109               |
| Krähen (Organe)               | 34                | -                 |
| Greifvögel (Organe)           | 5                 | 6                 |
|                               | (4 davon positiv) | (1 davon positiv) |
| div. Vogelspezies (Serologie) | 87                | -                 |
|                               |                   |                   |

In the wake of global warming and the associated spread of the primary WNV vectors, *Culex* mosquitoes, it is anticipated that the infection will spread in Austria following its first appearance in 2008. The further detection of WNV in a bird of prey with the same pathogenic lineage in 2009 may already be an indication of viral hibernation.

## SPORADIC ANIMAL DISEASES

Individual cases of the following animal diseases were detected in the reporting year.

97 cases of blackleg in cattle

- 3 cases of mange in sheep and goats
- 2 cases of viral haemorrhagic septicaemia in trout
- 1 case of infectious haemorrhagic necrosis in fish
- 1 case of varroasis in bees
- 122 cases of American foulbrood in bees



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