# *Farm animal reproduction: Reducing infectious diseases*

Proceedings from a symposium at
The Faculty of Veterinary Medicine, Jelgava, Latvia,
Januari 22-23, 2003

Vita Antane and Ulf Magnusson *(editors)*

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## Foreword

The Symposium "Farm animal reproduction: Reducing infectious diseases" at the Faculty of Veterinary Medicine in Jelgava, Latvia on January 22-23 is a part of the cooperative programme "Farm animal reproduction: Reducing infectious diseases and Conserving local genetic resources" between Estonian Agricultural University (EAU), Tartu, The Lithuanian Veterinary Academy of Lithuania, Kaunas, Faculty of Veterinary Medicine, Latvia University of Agriculture, Jelgava, and the Centre for Reproductive Biology in Uppsala, Swedish University of Agricultural Sciences. The cooperation is financially supported by *"Nya Visbyprogrammet*" at the Swedish Institute, Stockholm.

This symposium focuses on infectious diseases that may reduce farm animal fertility, because that can have a tremendous impact on the economical profit of animal production. In Sweden the productivity in the dairy industry has increased considerably after implementing control programmes for such diseases. Similar positive effect could be expected in the Baltic States, helping the livestock sector reaching a productivity similar to that in the Nordic countries. Also, several infectious diseases that pose a threat to animal health in general, may be transmitted at natural mating or by semen of artificial insemination. An efficient control on infectious farm animal diseases is a very important component in the agricultural politics in the EU. The disastrous effect of infectious farm animal diseases such as foot and mouth disease on economy and ethics is well known for everybody.

Here we have gathered speakers from all four countries, giving presentations on the biology of several infectious diseases in livestock as well as on control measures in the different countries. This assures a stimulating scientific and collegial exchange.

On behalf of the national programme-coordinators, Drs. Toomas Tiirats in Estonia, Henrikas Zilinskas in Lithuania and the under-signed we wish you a pleasant reading!

Jelgava and Uppsala, January 2003

*Vita Antane and Ulf Magnusson* (editors)

# Leptospirosis and Reproduction in Livestock

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Leptospirosis is bacterial disease that in animals is characterised by reproductive failure, such as abortions, stillbirths and birth of weak offsprings. It is also an important zoonosis, where for example veterinarians, abattoir workers, farmers and meet inspectors may be at risk. In this abstract, leptospirosis in pigs and bovines is discussed.

Leptospires were previously classified as the pathogenic *L. interrogans* or the saprophytic *L. biflexa* by serological typing on the basis of agglutination to serovar level. Presently, there are some 230 recognised pathogenic serovars. This antigenic classification has changed to a genetic that divide leptospires into different genomspecies (*L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, *L. noguchii*, *L. weilii*, *L. alexanderi*, *L. santarosai*, *L. meyeri, L. inadai* and *L. fainei*). However, for practical reasons the serovar is the basis of taxonomy.

Leptospirosis is transmitted directly or indirectly via infected urine. Direct transmission occurs through intact mucous membranes or skin cuts and abrasions. Infection via the vaginal route through infected semen is also possible. Indirect transmission occurs via contamination of the environment, for example through urine contaminated effluent, feed, water or soil. Following introduction and bacteraemia, leptospires localise in the kidneys and are shed in the urine, from weeks up to more than a year, depending on animal specie and serovar. Despite the large number of serovars that have been found there are usually only a small number that are present in a particular region. Most often they are associated with one or more animal species that inhabit the area and act as so called maintenance hosts. Transmission of infection among maintenance hosts is efficient and the incident of infection may be high. In contrast, incidental hosts are less important in transmission of infection as they rapidly eliminate leptospires from the kidneys, however these hosts may be important carrier of infection.

*Leptospira* infection may be introduced to a herd when new animals that carry leptospires are brought to the farm, through a contaminated environment or through contact with other infected animal species. The extent to which leptospires are transmitted within an animal herd depends on factors such as climate, population density, and degree of contact between maintenance and incidental hosts. One factor of importance for occurrence of clinical disease is the immune status of the herd. In a herd with waning immunity or in a previously uninfected herd, clinical signs may be seen in all age categories and cause substantial reproductive losses mainly due to abortions. In subsequent *Leptospira* infection or in areas with endemic infection, symptoms are usually restricted to non-immune females.

In pigs, *Leptospira* infection is most often caused by the serovars bratislava, tarassovi and pomona. These serovars are maintained by pigs and cause reproductive disturbances in the chronic phase of infection and may be shed in the urine for more than a year. Infection with serovar bratislava may differ from that of other serovars as venereal transmission play an important role in the spread of this particular serovar. Serovar bratislava may also persist in the oviduct and uterus of non-pregnant sows. Apart from these three serovars, there is a wide range of others that cause acute, severe incidental infection, which is followed by a rapid elimination of leptospires from the kidneys. In most cases *Leptospira* infection proceeds without clinical signs. In the acute stage of disease symptoms of anorexia, diarrhoea, jaundice,

haemoglobinuria and weakness are seen. This stage of disease coincides with the presence of leptospires in tissues. It has also been reported that if disease is caused by a strain of low virulence, or if the herd is infected endemically, clinical signs may be mild and overlooked. Reproductive disorders, such as abortions, stillbirths and birth of weak piglets, are associated with the chronic form of leptospirosis. Reproductive disturbances may be accompanied by fever, reduced milk production, haemoglobinuria and jaundice. There may be substantial economic losses due to abortions and death of weak piglets. Usually the reproductive performance in sows and gilts return to normal after clinical leptospirosis with reproductive disturbances. Abortions are usually recorded in the last trimester in pigs and is likely to result from toxic products that the dead infected foetuses release. It has been reported that birth of weak or dead piglets, due to leptospiral infection, may coincide with birth of normal piglets.

Bovine leptospirosis most often result from infection with the serovars hardjo (type hardjoprajitno or hardjobovis), pomona or grippotyphosa. Cattle are usually regarded as maintenance host for serovar hardjo. As in pigs, infection may result from a wide range of serovars. In general, infection with maintenance host serovars is associated with a high degree of subclinical infection in non-pregnant animals, whereas chronic infection cause reproductive failure and infertility in cows an heifers. Losses due to abortion may be substantial (up to 30% abortions). Infection with incidental serovars may cause acute and severe disease with clinical signs of fever, anaemia, haemoglobinuria, jaundice, pulmonary congestion and eventual meningitis. In lactating cows acute leptospirosis is seen as transient fever with a substantial drop in milk production. This syndrome is called the "milk drop syndrome" in which the udder is soft and flabby, the milk is yellow and contains thick clots. Also, calf mortality may be high. In the chronic form of leptospirosis reproductive failures are recorded, such as abortions, stillbirths, birth of weak calves and infertility of the cow. Reproductive failure are mainly recorded during the last part of pregnancy. The period of urinary shedding is usually shorter for cattle compared with pigs.

Diagnosis requires laboratory procedures and the methods used are either serological tests or demonstration of leptospires in clinical and autopsy samples. The most widely used serologic test is the Microscopic Agglutination Test (MAT). This test is specific for the infecting serovar, although cross-reactions may be recorded against other serovars within the same serogroup. Therefore, local *Leptospira* isolates should be included as antigens in the MAT, or serovars that are known to infect the particular animal specie elsewhere. The MAT has been considered as confirmative for a positive diagnosis in individual animals if a rising titre is recorded in paired samples or if the initial titre is ∅1:400. However, the MAT is of limited value in chronic infections as low titres may remain for years after infection in individual animals and, therefore, the test may be considered primarily as a herd test. Also, infection with species-adapted strains may yield antibody titres less than 1:100. This may for example be seen after serovar bratislava infection in pigs. Leptospiral antibodies have been shown to appear in the circulation approximately one week after infection and the highest titres have been recorded ten days to three weeks after infection. Another serologic test that are used in livestock is for example Enzyme Linked Immuno Sorbent Assay (ELISA).

Demonstration of leptospires in internal organs or body fluids is confirmative for diagnosis of leptospiral infection. In the acute stage of the disease, organisms can be found in liver, lungs, brain or blood, cerebrospinal-, thoracic-, and peritoneal fluids, and in the chronic stage of infection mainly in kidneys, urine, and genital tract or in aborted foetus and foetal membranes. Different methods to demonstrate leptospires in tissue have been described, such as direct examination by dark-field microscopy and light microscopy after appropriate

staining, immunofluorescence, immunohistochemistry, culture, or the polymerase chain reaction (PCR). Isolation by culture is effective for epidemiological studies when an infecting serovar needs to be identified, however, it is a time-consuming and fastidious method. Before culture, clinical specimens are homogenised, diluted and inoculated in semisolid medium containing antimicrobial agents to reduce contaminating flora, and incubated for up to 26 weeks and read weekly for the presence of leptospires.

In order to reduce infection a combination of measures based on management, treatment and vaccination can be implemented. On farm level, management procedures may, for example, include measures to reduce the rodent population and contacts between domestic and wild animals, to keep different animal species and newly introduced stock separate from the rest of the herd. To reduce urinary shedding, or eventually eliminate renal leptospirosis, animals may be treated with streptomycin, oxytetracycline, tylosin or erythromycin. Furthermore, antimicrobial agents in the feed, such as oxytetracycline or chlortetracycline, may reduce clinical signs, but will not eliminate carriers. Vaccination may reduce clinical signs and leptospiruria in a herd, but will not completely eliminate urinary shedding. Immunity to clinical disease following vaccination usually lasts longer (six months to a year) than immunity to infection. As immunity is serovar specific, a vaccine has to include the infecting serovars within the region.

# *Neospora caninum* infection in cattle

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## Introduction

In the early 1980's a new syndrome was described in young dogs with paralysis.[6] The syndrome was proven to be caused by a hitherto unknown intracellular coccidian parasite that was given the name *Neospora caninum*.[19] Later, the parasite has been found in a variety of animal species such as cattle, goat, sheep and deer. Today neosporosis is recognized as a major cause of bovine abortion around the world. Several reviews of *N. caninum* and neosporosis have been written over the years, e.g.[3,16,17,20], and last year a redescription of the parasite was published.[18]

## Life cycle and transmission routes

*N. caninum* has a two-host life cycle including intermediate and definitive hosts. The life cycle is not fully understood but it is known that dogs and cattle play important roles. Dogs are the only proven definitive host of the parasite and can shed oocysts by its faeces after ingestion of infected tissues.[26] Several animal species including e.g. cattle, water buffaloes, pigs, red foxes and monkeys serve as intermediate hosts. They can be infected by ingestion of oocyst-contaminated water and feed, or by eating tissues of infected animals. See Figure 1.

In cattle, congenital infection, i.e. transmission of parasites from a cow to her foetus during pregnancy, is considered the dominating route of infection. This assumption is supported by investigations undertaken in Europe and USA showing that up to 78-95% of calves born to infected cows are themselves infected.[12,29] Congenital transmission can occur during consecutive pregnancies, and congenitally infected heifers can later transmit the parasite to their own offspring.[8] Thus the parasite can persist for a long time in an infected herd without involvement of a definitive host. Cattle may also acquire *N. caninum* infection through ingestion of oocysts that are shed in the faeces of acutely infected dogs.[14,26] Intake of contaminated colostrum or milk has led to infection in young calves in experiments[38] but whether this occurs naturally remains to be shown.

## Clinical effects of *N. caninum* infection in cattle

Non-pregnant *Neospora*-infected adult cattle show no clinical sign of infection. A majority of pregnant infected cows, however, transmit the infection to their foetuses and this results in abortion, birth of a weakly calf, or birth of a clinically healthy but persistently infected calf. The mechanism by which the parasite is transmitted from dam to foetus is unknown, as are the factors which determine the outcome of infection.

Today neosporosis is recognized as a major cause of bovine abortion in many parts of the world.[3,4,34,37,40] Both acutely and persistently *N. caninum* infected cows are more prone to abort than are non-infected cows.[8,25,35,41] Infection increases the risk of abortion with 2-7 times[13,27,30,35,41], and congenitally infected heifers seem to be most at risk to abort during their initial pregnancy.[35] Repeat abortion in *N. caninum* infected cows occur[5,35,41] even though it is not known how common this is. In one investigation it was found that congenitally infected

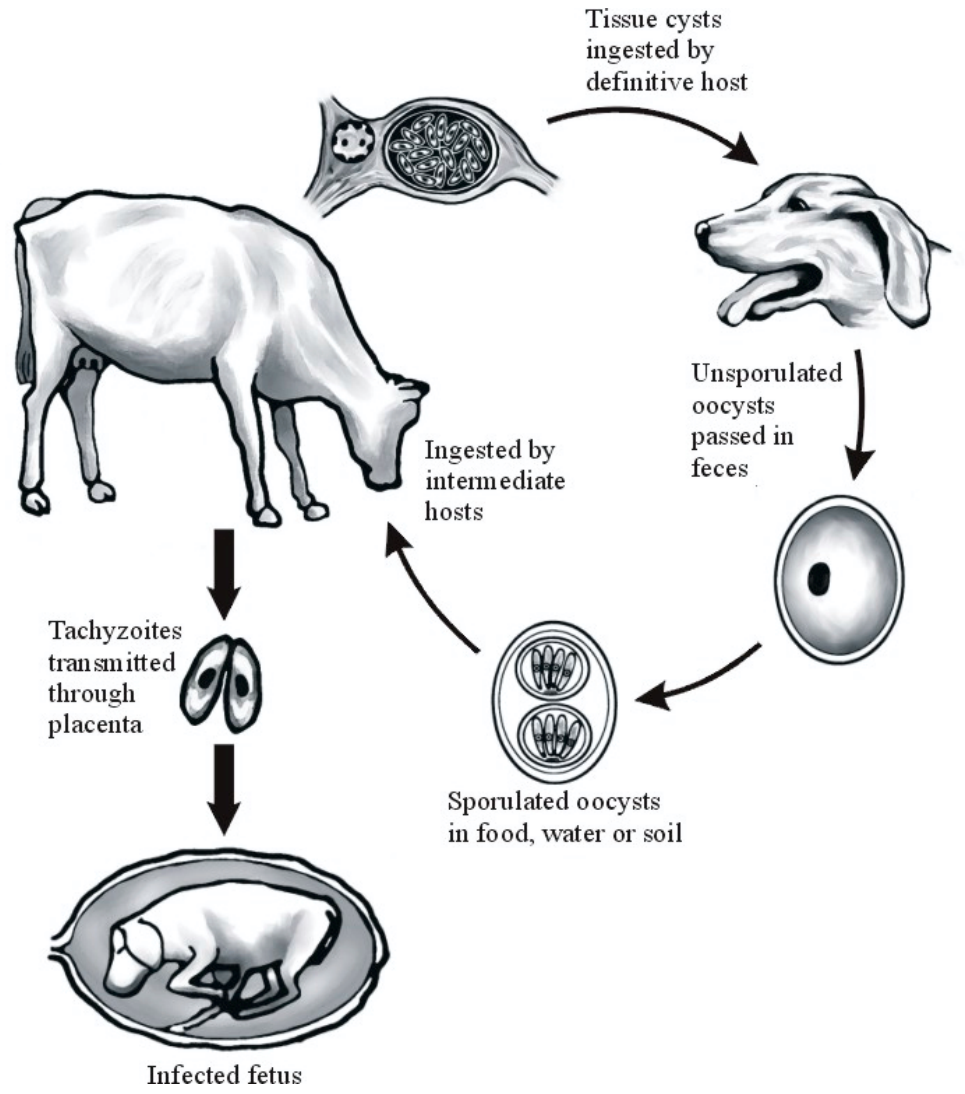


Figure 1. The life cycle of *Neospora caninum* (Stenlund, 2000. PhD thesis, Swedish University of Agricultural Sciences, Uppsala Sweden).

cows that aborted had a 5.6 times increased risk for a subsequent abortion than infected cows that had not aborted previously.[35]

*N. caninum* induced abortion can occur throughout pregnancy and may include stillbirth at full time but abortions at 5-7 months of gestation is the most common.[4,27,28] The fact that the parasite is transmitted from cow to calf during subsequent pregnancies and that repeat abortion do occur shows that the maternal immune response is unable to prevent the foetus from infection. However, investigations in *Neospora* infected cattle herds indicate that chronically infected cows have a lower risk to abort during a point-source exposure to the parasite compared to previously non-infected animals.[23,25] This suggests that cows can acquire a protective immunity against *N. caninum* induced abortion. That herds with a predominance of congenitally infected cows have somewhat increased annual abortion rates compared to control herds[8,15,41] whereas a point-source infection causes a large number of the pregnant cows in a herd to abort[15,23,25] support this assumption.

In addition to causing abortion, *N. caninum* infection seem to have a detrimental effect on bovine pregnancy rate and milk production.[33,36,39]

**Diagnosis**

Histology, immunohistochemistry, polymerase chain-reaction techniques (PCR) and isolation of the parasite in cell culture can be used on post mortem samples to confirm *N. caninum* infection.[22] In a live animal, presence of antibodies indicates that it is infected and is most likely still carrying the parasite. A variety of serological assays including enzyme linked immunosorbent assays (ELISA), indirect fluorescent antibody assays (IFAT), agglutination tests and immunoblotting have be used to demonstrate presence of *N. caninum* antibodies.[11] ELISAs can also be used to detect antibodies in foetal fluids, individual milk and bulk tank milk. Tests kits for *N. caninum* antibodies in serum are today commercially available. The specific IgG antibodies can persist at high levels for a long time and can fluctuate during pregnancy. Therefore, the level of IgG antibodies or demonstration of rising antibody titres cannot be used to estimate if an individual suffers from acute or chronic infection. An avidity ELISA has been described that overcome this drawback by measuring the avidity (binding strength) of antibodies in serum.[9,10] The basis for this assay is that the strength of binding of antibodies to *N. caninum* increases with time after infection.

**Bovine *Neospora*-infection in the Nordic countries**

*N. caninum* has been demonstrated in aborted fetuses and stillborn calves in Denmark and Sweden[1,2,21], and the first European bovine isolate of the parasite (Nc-SweB1) was obtained in Sweden in 1995.[32]

In Sweden it is estimated that 2% of the dairy cows have antibodies to *N. caninum*.[7] This low prevalence of *N. caninum* infection in Swedish dairy cattle is favourable in relation to possible future control programs. No investigations of the overall infection rate in cattle have been performed in the other Scandinavian countries.

In both Sweden and Denmark *N. caninum* infection has been shown to be associated with bovine abortion. In a Swedish study, 7% of aborting cows in herds considered as having abortion problems was shown to have antibodies to the parasite.[7] These animals were also investigated for the presence of antibodies to bovine virus diarrhoea virus (BVDV) and 42% were found seropositive, indicating that there might be concurrent effects of *N. caninum* and BVDV.

In Denmark, the prevalence and distribution of seropositivity towards *N. caninum* were investigated in 31 dairy herds, half of which had experienced abortion problems.[24] Seropositive animals were found in both groups of herds and the seroprevalence ranged from 1% to 59%. The abortion risk was significantly increased in seropositive animals and in $\geq$2nd gestation cows, whereas reproductive performance and culling risk were not affected by serostatus.

Detailed investigations are being done in infected Swedish dairy herds to clarify transmission routes and which impact *N. caninum* has on reproduction. The seropositivity in the infected herds varies considerably with seroprevalences between 5 and 87%. In Sweden as in most other countries, congenital infection is the dominating route of parasite transmission. However, horizontal transmission also occurs in many herds. The long-time effect of *N. caninum* infection on the abortion rate varies both between herds and over time.[8,31,33] Most *N.*

*caninum* infected herds that are known have been diagnosed when they experience acute problems with abortions. Thereafter might follow a period (one to several years) with sporadic abortions during which the overall abortion rate is only somewhat higher than the 2-4% expected in Sweden, before the abortion rate again increase. The reason for these differences between calving seasons is not known and the ongoing Swedish *Neospora* research are dealing with these questions.

In the other Nordic countries bovine neosporosis has been less in focus. In Finland, 1 out of 40 investigated abortion problem herds had *N. caninum* seropositive cows in 1998 (<http://www.mmm.fi/el/julk/eltaud98en.html> 6 December 2002). In Norway, at least one seropositive aborting cow has been identified (Fossen & Björkman, unpublished finding). As far as I am aware, no cattle have been tested for *N. caninum* infection in Iceland and the Faeroe Islands.

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# Mastitis bacteriological diagnosis and its specific prophylaxis in dairy cows

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Although public health and welfare primarily depends on animal productivity increase the quality improvement is even more dominant. There are various obstacles for success in animal production. Many diseases affect dairy cows, particularly, udder inflammations, which are the most spread not only in Latvia but also in other countries. According to our investigations and knowledge about 5-6 % of cows suffer from acute mastitis and up to 30% from subacute mastitis. Mastitis is registered in all type farms and in countries with developed milk production this disease makes up considerable social and economic problem because of hazard to both young calve and human, particularly, children health. Milk obtained from dairy cows often contains pathogenic microbes and their metabolic substances - toxins, including thermostable toxins, antibiotic residues after treatment as well as other groups of noxious adulterants making milk substandard and bad quality. The described situation is due to underestimation of real size of mastitis problem, a lot of misbelieved and recondite questions, not adequate use of milking system (often due to money saving perceptions for a long time no check of vacuum level or pulsator function) resulting in damage to cow udder tissues, often arise disturbances of milk ejection reflex, milk withhold in udder, large milk amount remaining in udder which contributes to inflammation start and development in mammary gland. Calves born from cows with mastitis do not receive adequate amount of biologically necessary colostrums. In the milk with a high somatic cell count (higher degree of inflammation) is increased concentration of amino acids, there are increases of all amino acids, but arginine is increased significantly ($p<0.01$). The natural resistance of such calves is depressed, therefore they often suffer from digestive tract diseases. Afterwards these calves retard in growth and development, even when adult they have lower productivity and are more susceptible to various diseases, they have lower fertility.

Prolonged use of antibiotic substances in treatment of mastitis makes the pathogens adapted to them in udder tissues, more than 85% of isolated pathogens show some resistance against antibiotics. At the same time changes also reactivity of whole cow body and local reaction of tissues, to some degree favorable for maintain of inflammation (during the $60^{th}$ and $70^{th}$ the most spread mammary pathogen was Streptococcus agalactiae, afterward during $80^{th}$ equal spread of Streptococcus agalactiae and Staphylococcus aureus, Starting with $90^{th}$ the most spread mastitis pathogen is Staphylococcus aureus), there are changes in typical mastitis clinical picture, typical development. Often mastitis is mild clinical or subclinical, but chronical with gradual destruction of glandular tissue and increased sensibility of cow body. In the practical treatment of mastits still is wide use of antibiotic substances, not rare without adequate justification, because often there are no bacteriological investigation results and there is no knowledge about its eventual susceptibility against chosen antimicrobial. Not rarely antibiotics and other inhibitory substances occur in the milk and are a hazard for consumer health as well as are obstacle for technology process in milk processing plants, because such a milk is not suitable for making high quality milk products.

There are a lot of old suggestions, contradictory arguments and not clear statements about treatment and control of dairy cow mastitis and this is one of reasons for wide spread of different degree mastitis among dairy cows, which are the cause for great economic loses for

animal owners as well as are epidemiologicaly dangerous. Therefore in the complex solution of mastitis problem important role has timely diagnostic of mastitis pathogen, particularly, of subclinical mastitis, which are found from regular monitoring of milk somatic cell count. Count of somatic cells in milk of each cow should be estimated on monthly bases either by direct counting (analyzer "Somacount") or by non-direct method with some of test solutions (California mastitis test, Dosil test etc.). If the count of somatic cells is increased, aseptical (before taking sample teat end is disinfected with cotton soaked in 70% alcohol) milk sample from given cow is taken in sterile tube with sterile cup for bacteriological diagnostic. The sample with descriptive document is sent to the laboratory for isolation of mastitis pathogen or investigated in practice veterinary laboratory.

In the laboratory we inoculate milk on pepton agar plate. After incubation we estimate growth on the plate, shape of colonies, biochemical properties, microscopic appearance and basing on all characteristics made the conclusion. We use also selective media for pathogen identification (for staphylococci – blood peptone agar, Baird Parker agar, Mannitol salt agar, milk agar; for isolation of streptococci – blood peptone agar, KF streptococci agar; for gramnegative pathogens – Endoagar, MacConkey agar, Levin agar, Klark media, Simons agar and Hiss sugar) and BBL CRYSTAL$^{TM}$ Identification Systems. After isolation and identification of pathogens we evaluate their susceptibility against usually used antibiotics in treatment of udder inflammation. As soon as possible after identification of pathogen and choose of most effective curative means treatment is started. After course of treatment we repeat sampling of milk and investigate bacteriologicaly. If the pathogenic microorganism is still present in milk, treatment course should be repeat and if two or three therapeutic periods are unsuccessful the cow is considered for culling. Investigations of udder secret from mastitic cows does show that on average in our country most of udder inflammation is caused by Staphylococcus aureus, 48,5%, next is Steptococcus agalactiae, 26.4%, followed by Escherichia coli, 11.9% etc. According to type of farm Staphylococcus aureus is the dominant in 1-5 cow herds, 56.1%, and in big farms with more than 100 cows, 48.0%. The highest spread of Streptococcus agalactiae is in medium size farms with 6-100 cows, 44.6%.

Meny farmers are going to be organic-biological producers of ecologicaly wholesome milk and a lot of antibiotic and chemotherapeutic medicines will be no more allowed for treatment of animals, also for treatment of udder inflammation. This was a reason to work on new strategies against mastitis problem, to work out method for production of specific antigens for immunization of animals, to keep them away from Staphylococcus aureus caused udder inflammations.

For production of antigen we use 5-6 Staphylococcus aureus isolates from acute clinically ill cows. All the isolates are pathogenic and produce both alfa and beta toxins. Antigens are produced in the laboratory according to early proven method, and experimental batch is evaluated in volunteer farms, where Staphylococcus aureus mastitis is actual problem. Dose of antigen is $2.5*10^9$ microorganisms per ml and it is applicated two times with 12-14 day interval. Three weeks after immunization we take blood samples from immunized cows and detect antibody level in the serum by agglutination test and imunodifusion test. For following up antibody levels we take blood samples from cows on monthly intervals till they disappear in all animals. Results show that immunization of cows with experimental antigen produced levels of specific antibiotics as high as 1:64 till 1:1024 and all the animals immunizes twice by 12-14 day interval remain high for next five months, but during sixth month already 11.8% of immunized animals show lack of specific antibodies. After immunization serum titre decrease each month: during first month is only slight decrease ($p<0.05$), but during next

months significant decrease ($p<0.01$-$0.001$). To maintain continuous level of specific antibodies in the serum, there is need for reimmunization in the 5$^{th}$ month, also as double boost by 12-14 day interval. This increase titres of antibodies in blood serum of all animals for next 5 month, but during 6$^{th}$ month 8.6% of animals had no more specific antibiotics. To maintain continuous level of specific antibodies against Staphylococcus aureus and to protect cows against mastitis there is necessary to repeat immunization each five month. Bacteriological analyses of milk from immunized cows do show no Staphylococcus aureus. Immunization of cows with Staphylococcus aureus antigen not only works prophylacticaly against udder inflammation, but also decreases somatic cell count in the milk of experimental animals compared with nonimmunized control group. For cows with highest somatic cell count before immunization, decrease afterwards is significant.

**Conclusions.** (1) All the cows and pregnant heifers immunized by experimental Staphylococcus aureus antigen develop specific antibodies in serum titres 1:64 till 1:1024;
(2) To maintain continuous level of specific antibodies against Staphylococcus aureus and to protect cows against mastitis there is necessary to repeat immunization each five month;
(3) Immunized cows and pregnant heifers after calving have lower somatic cell count in milk, than non-vaccinated;
(4) Bacteriological analyses do not show any Staphylococcus aureus in milk from immunized and reimmunized cows.

# The State Surveillance Programme of Animal Infectious Diseases in Artificial Insemination Establishments (Stations, Sections and Points) and main Problems in Latvia

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Some years ago in the Republic of Latvia were working 11 artificial insemination establishments (Lielvardes, Daugavpils, Kandavas, Gulbenes, Liepajas, Kuldigas, Alojas, Valmiera district "Beites", Kurzemes, Siguldas and Latgales), where were located about 65 bulls. At this moment in Latvia working 3 Stock register and Artificial insemination stations and 3 artificial insemination points.

Stock register and Artificial insemination stations (SRAIS):

1. Latgales SRAIS (LV 010W) - 26 certificated bulls and 5 certificated boars.
2. Siguldas SRAIS (LV 009W) - 37 certificated bulls.
3. Kurzemes SRAIS (LV 007W), which central location is Tukuma district Jaunpils (LV 007W), with two sections - in Kandava and Liepaja. In Jaunpils there are 26 certificated bulls and 18 certificated boars; in Kandava section - 23 certificated boars; in Liepaja section - 12 certificated boars.

Artificial insemination points (AIP):
1. Preilu district, company "Regīna" - 4 certificated boars.
2. Riga district "Ulbroka" - 17 certificated boars.
3. Dobeles district " Tērvete" - 9 certificated breed - stallions.

The accordance to requirements and order of registration of animal artificial insemination stations and embryo transplantation establishments are determined by the rules of the Republic of Latvia No. 459 from 08.11.01. on "The accordance requirements and the order of registration of animal artificial insemination stations and embryo transplantation establishments".
Food and veterinary service (FVS) basing on application of SRAIS and on results of FVS territorial unit inspection assigns veterinary surveillance number for production and realization of certificated breed animal bio products.
FVS has assigned the numbers of veterinary surveillance to all above-mentioned three SRAIS.
The order how there must be certificated breeders of cattle, pigs, sheep, goats and horses, their bio products and embryos intended for transplantation is determined by the rules of Minister Cabinet of the Republic of Latvia No. 211 from 22.05.01. "The order of certification of breeders, their bio products and embryos intended for transplantation".

The order how is certificated natural persons which are carrying out the evaluation and control of different animal species, animal surveillance, artificial insemination, processing of breeders and embryos, realization of breeders bio products and embryos, as well as import and export of breed material, is determined by the rules of Minister Cabinet of the Republic of Latvia No. 194 from 15.05.01.
SRAIS (sections, point) buy in animals from bull dam breeding establishments that are free from any infectious disease. Before movement from breeding farm animals, depending on specie, must be tested on infectious diseases that are laid down in " The state surveillance action plan of animal infectious diseases".
If breeders are buy in from abroad then they must be tested before movement on diseases determined by Food and veterinary service in accordance with veterinary (health) certificate.
Breed animals are moved to SRAIS (section, point) observing rules of animal transportation (Rules of Minister Cabinet of the Republic of Latvia No. 309.).
Before animals are placed in SRAIS (section, point), they must be determined on quarantine during 45 days outside the territory of SRAIS (section, point), the place of quarantine must be co-ordinated with the FVS territorial unit and during this time breeders are tested on infectious diseases laid down in " The state surveillance action plan of animal infectious diseases" - investigation results must be negative. During quarantine it is prohibited to take semen from breeders.
Breeders of artificial insemination stations, sections and points every year must be tested on diseases in part A determined by " The state surveillance action plan of animal infectious diseases":

Certificated bulls on:

- ∉ Tuberculosis - once a year
- ∉ Brucelosis - once a year
- ∉ Enzootic leucosis - twice a year
- ∉ Campylobacteriosis - twice a year
- ∉ Trichomonosis - twice a year
- ∉ Bovine virus diarrhoea - twice a year
- ∉ Leptospirosis - twice a year
- ∉ Infectious rhinotracheitis/ infectious pustular vulvovaginitis - twice a year
- ∉ Hlamidiosis - twice a year
- ∉ Paratuberculosis - twice a year

Certificated boars on:

- ∉ Tuberculosis - twice a year
- ∉ Brucelosis - twice a year
- ∉ Aujeszky's disease - once a year
- ∉ Porcine reproductive and respiratory syndrome - once a year
- ∉ Atrophic rhinitis of swine - once a year
- ∉ Porcine parvovirusal infection - once a year
- ∉ Leptospirosis - once a year

Certificated breed stallions on:

- ∉ Dourine - once a year
- ∉ Equine infectious anaemia - once a year
- ∉ Glanders - once a year
- ∉ Rhinopneumonitis - once a year

- Infectious arteritis - once a year

Serological testing of certificated bulls in 2002

| Disease | Siguldas AIS | | Kurzemes AIS | | Latgales AIS | |
|---|---|---|---|---|---|---|
| | 1x | repeated | 1x | repeated | 1x | repeated |
| Tuberculosis (allergic) | 37 | 37 | 33 | 31 | 25 | 25 |
| Brucelosis | 37 | 37 | 33 | 31 | 25 | - |
| Brucelosis | 37 | 37 | 33 | 31 | - | - |
| Hlamidiosis | 37 | 37 | 33/+1 | 31 | 28/+1 | 25 |
| Leptospirosis | 37 | 37 | 33 | 31 | 28 | 24 |
| Enzootic bovine leukosis | 37 | 37 | 33 | 31 | 28 | 24 |
| Bovine virus diarrhoea, (Ab) | 37/+5 | 37/+5 | 33/+1 | 31/+2 | 28/+3 | 28/+2 |
| Bovine virus diarrhoea, (Ag) | 37 | - | 5 | 3 | 3 | 2 |
| IRT-IPV | 37/+22 | 37/+22 | 33/+4 | 31/+4 | 28/+2 | 24/+2 |
| Paratuberculosis | 37 | 37 | 33 | 31 | 28 | 24 |
| Trihomonosis, Microsc. | 37 | 37 | 33 | 31 | 24 | 24 |
| Campylobacteriosis, Microsc. | 37 | 37 | 33 | 31 | 24 | 24 |

In all SRAIS till the year 2001 intended to carry out the vaccination of bulls against IRT/IPV. In 2003 it is planned to carry out additional testing of bull semen on BVD and IRT/IPV virus presence.

Serological testing of certificated boars in 2002 during ten months

| Disease | Jaunpils AIS | Latgales AIS | Kandavas section | Ulbrokas AIP |
|---|---|---|---|---|
| Tuberculosis (allergic) | 38 | 22 | 4 | 57 |
| Brucelosis | 38 | 22 | 4 | 57 |
| Leptospirosis | 26 | 6 | - | - |
| Parvovirusal infection | 26/+9 | 7/+4 | - | 28/+4 |
| Porcine reproductive and respiratory syndrome | 26 | 30 | 4 | 50 |
| Aujeszky's disease. | 26 | 30 | 4 | 57 |

Evaluating results of breed animal laboratory testing we have come to conclusion that at the moment in artificial insemination stations there are problems regarding bulls caused by two diseases - infectious rhinotracheitis/infectious pustular vulvovaginitis (IRT/IPV) and bovine virus diarrhoea (BVD). From the year 2001 it is prohibited the vaccination of bulls against IRT/IPV. All bulls which were laboratory tested and detected antibodies against IRT/IPV are

vaccinated till 2001. The part of these bulls have already been eliminated and soon will be eliminated the other ones. Bulls in which blood were detected antibodies against BVD virus are re-tested on presence of antigens. After testing of bulls antigens of BVD were not detected. The vaccination of bulls against BVD is not allowed. Further there will be not allowed the presence of bulls in SRAIS that reacted to antibodies of BVD agents. All boars that showed positive reaction to antibodies of porcine parvovirusal infection are vaccinated with complex vaccine against leptospirosis and parvovirusal infection. This vaccination for boars of artificial insemination establishments are allowed.

At present no one of all Latvia artificial insemination establishments has rams and he-goats intended for breeding. They are in some separate farms. The main problem in ovine herd is disturbance of Maedi-Visna disease agent, but in caprine herd - the agent of Caprine arthritis/encephalitis.

Food and veterinary service has elaborated the rules of BVD, IRT/IPV, Maedi - Visna disease and Caprine arthritis/encephalitis prophylaxis and eradication.

# "FRISKKO" – The Animal Health Programme in Swedish Dairy Production

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Swedish dairy farmers have a great need for an increased emphasis on systematic preventive animal health actions. The two main reasons for this are:

1. The increased interest among consumers in animal health and welfare issues.
2. A continuously stretched production economy due to heavy market competition, making it urgent to prevent costly disease problems.

The top five expectations among Swedish consumers on Swedish dairy producers and their products entirely concern food safety and animal welfare aspects. They demand:

- Products to be free from salmonella
- Good quality of feedstuff to the cows
- Well-managed cows
- No hormones except for treating diseased cows
- No antibiotics except for treating diseased cows

Calculations made by the Swedish Dairy Association show great variations in health costs between herds. Costs may differ by as much as 0.6 SEK per kg milk. In a stretched economical situation, a good herd health status is a prerequisite in order to reach an acceptable net income. A reasonable estimate is that by applying consistent preventive measures, the result on most farms could be improved by at least 0.1 SEK per kg milk.

The above background explains why the Swedish Dairy Association and its local livestock societies have been engaged in the development of a system for animal health care in co-operation with scientific institutes and veterinary practitioners (1, 2). Testing different models has resulted in a current concept called FRISKKO (*healthy cow*) (3).

The aims of FRISKKO are:

- Improved animal health
- To provide the food industry with safe and ethically produced products
- Better production economy in the herd by reducing costs for poor animal health.

The effect of the programme has been evaluated in terms of herd health and herd economy in two different studies. The result has been very encouraging, showing that the approximately 60 affiliated herds had earned 600 SEK per cow and year, or 0.07 SEK per kg milk, compared to 450 herds not affiliated to the programme. The result was attained mainly due to lower disease costs, higher milk yield, and improved breeding of heifers (4).

The general procedure when a herd is affiliated to FRISKKO is to start with the *Basic service* for at least two years. The *Basic service* includes two scheduled visits annually by the veterinary practitioner, one of which together with a veterinarian from the local livestock society specialising in animal health. A comprehensive herd investigation is made, including the entire chain from calf to cow. Key health parameters reviewing the herd health status in

comparison with other herds and over time is presented. A simple assessment of economic losses caused by health disturbances is also made. In September 2002 the Swedish Dairy Association introduced a new unique system with health indicators at herd level in all milk-recorded herds. These indicators can be ordered by any milk-recorded herd and give a broad comprehensive analysis of the health situation in the particular herd. The values are calculated continuously with reference values and give rapid signals whenever problems arise. Changes over time with reference values are also shown.

The health indicators are divided into six problem areas:

- Udder health
- Calf and young stock health
- Metabolic disorders
- Other diseases
- Culling pattern
- Fertility

The indicators are extremely useful in the work with animal health problems at herd level and are expected to be an efficient instrument in the supervision of the herd health.

After a scheduled visit, findings and advice are always documented and a consultative letter is sent out (stating priority orders if requested). All advice is given in communication between extension staff and veterinarians.

In addition, a periodical (”News on Animal Health”) directed to farmers, is distributed quarterly. If required, affiliated herds get free autopsies and certain free laboratory analyses. Access to subsidised vaccines in the case of outbreak of ringworm in the herd is also provided for. Great emphasis is put on record-keeping of drugs and agents.

During a scheduled visit, the need for more intensive support than what is included in the *Basic service* may be identified. *Extended services* may then be offered to the farmer. These are tailored to suit the individual demands/problems:

Problems with udder health or mastitis may lead to dynamic measurements of vacuum during milking, including studies on milking hygiene and routines, performed by advisors on milk quality from the local livestock society and/or from the local livestock society.

Problems with the health of calves and young stock may lead to an investigation of the calf and young stock situation performed by the local livestock society.Problems with the claw health may lead to an investigation, including record-keeping of disorders, performed by private hoof trimmers parallel to trimming.

Problems with metabolic disorders may lead to a detailed investigation of feeding routines and feedstuff composition performed by the extension staff at the local livestock society.
Problems with the fertility may lead to frequent relevant service activities performed by the local livestock society or by the veterinary practitioner.
On farms with a balanced situation and few or no identified health problems, the number of visits is minimised to one annual *Follow-up,* normally performed by the veterinary practitioner. In case of problems, the herd is recommended either to join the *Extended service*

or to have a joint visit within the *Basic service* together with the veterinary officer from the local livestock society.

The main characteristic of FRISKKO is its focus on the farmer and the herdsman as being the key elements of a successful herd health situation. The comprehensive picture of the herd health status given both to the farmer, the veterinary practitioner and the advisory staff is also a prominent feature of the programme, as is the formation of a network between the veterinarian specialising in animal health, the veterinary practitioner, the advisors on feeding and breeding, the claw trimmers, etc. A third predominant quality of FRISKKO is the endurance. The system involves long-lasting actions and advice built on solid preventive knowledge, rather than putting out fires when a problem has already developed.

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# BVDV Control in Sweden

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A scheme aimed at eradicating BVDV in dairy and beef herds has been in place in Sweden since September 1993. It is coordinated by the Swedish Dairy Association, with financial support from the Board of Agriculture (BA). Subsidies are mainly directed towards infected herds, so monitoring of non-infected herds is mostly paid for by the farmer's themselves.

Initially, affiliation was voluntary. However, since mid '97 the dairy industry has required that all its suppliers should participate in the scheme. In '99, the beef industry followed. On 1 June 2002, a decree was issued by the BA that made the scheme compulsory for those that had not yet subscribed (only a handful), and indirectly (through repercussions on the regulations of the voluntary scheme) put more restrictions on animal movements. Today, animals from non-certified herds can, in practice, only be sold to slaughter.

Scheme monitoring is by testing for antibodies in bulk tank milk, in pooled milk from 5-10 primiparous cows or in individual serum samples from 5-10 young stock over 12 months of age. BVDV certification requires that a herd is sampled twice with a 7 months' interval with an approved result. Herds that have had high risk contacts within one year prior to affiliation need to have 3 approved tests. Annual re-testing is required to maintain the status, but in order to *trade* animal as free from BVDV-infection, or to access common pastures, exhibitions and similar, the latest re-test with approved result must have been made within the last three months (4 months for herds monitored by bulk milk). All field work is carried out by veterinarians and technicians at the regional livestock associations in collaboration with state employed and private large animal practitioners. Samples of bulk milk is collected by the milk quality laboratories.

The prevalence of dairy herds with bulk milk antibody levels indicative of ongoing infection has decreased from 52% in April 1993 to 5% by the end of 2001. Today (3 Dec. 2002) 652 herds are under investigation (219 dairy and 433 beef). A majority of those are free from the virus and awaiting to resume scheme monitoring. During the course of the scheme, around 3,500 herds have been actively cleared from the infection.

The annual incidence of new, confirmed infections (positive virus isolation in a herd previously certified as being free from BVDV) has decreased from 6 to 2 promille between 2000 and 2002 (up to Dec.). In numbers, there have been 50 new cases in ~24000 herds in 2002 (up to December). (Note that as this measure of incidence is based on identification of PI animals, the time point when virus was introduced into those herds is by nature at least 12 months back in time).

Since January 2001, herds that become infected after certification have been subjected to an investigation aimed at identifying the cause of the breakdown. These reports indicate that a majority of new infections are caused by intentional or unintentional non-compliance with existing regulations. This simply emphasises how the awareness about BVDV issues needs to be constantly reiterated. Approximately 30% are suspected to be a result of indirect transmission, something seen relatively more today as the major routes of between-herd transmission are under control.

The main objective during the final phase of the eradication is to minimise the time from detection of new infections to a finalised virus clearance. Measures are being instituted to shorten the time from detection to action, and to improve the efficiency by which herds are cleared from the infection. For the future it is, among other things, desirable to find more cost efficient ways to monitor beef herds. Also, faster early warning systems would be welcomed, in particular for beef herds.

# The Swedish Eradication and Supervision Programmes for Enzootic Bovine Leucosis (EBL) and Infectious Bovine Rhinotracheitis (IBR-IPV).

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Swedish Dairy Association is a free-standing national organisation for the regional livestock societies in Sweden. Swedish Dairy Association has been commissioned by the Board of Agriculture to be the authority responsible for the Swedish EBL and IBR eradication programmes. Swedish Dairy association is in charge of the overall administration of this programmes, involving financial aspects, organization, issuing of regulations within the programmes, information and contacts with authorities, the livestock and dairy industry and research institutions.

## The Organization of the EBL and IBR Programmes

Administration and computer processing are handled by Swedish Dairy Association. The basis of the programmes is the computer system in which data on all herds included in the programmes are stored and processed according to the programme regulations. The data base controls the measures taken in the individual herds by issuing documents needed for sampling as well as accounts and planning material. These documents are sent to the respective livestock societies.

The documents needed for sampling include case records and bar-code labels used to identify all samples sent to the National Veterinary Institute (SVA) for analysis.

The analysis results are transferred from SVA to the data base via telecommunication. These results are reported to the livestock societies once a week. In this way the herds can have a written report of the analysis results within one to two weeks. In urgent cases the result of an analysis is generally available after tree days.

The organization of the field work is handled by the regional livestock societies.

The field work is carried out by technicians employed by the livestock societies under supervision of veterinarians or by the local veterinary practitioners.

All analysis of samples is done by the National Veterinary Institute (SVA) , Uppsala.

## Measures Taken Within the EBL Programme

1. The programme aims at improving the hygienic standard in order to prevent the spreading of bovine leucosis virus (BLV) between and within herds. This is accomplished by means of general information to all people who in their work get in contact with dairy or beef cattle herds.

2. As soon as possible after joining the programme, blood or milk from all animals older than 12 months are sampled and tested for antibodies against BLV. The herd must slaughter all BLV-infected animals within 2 months because when you have infected

animals present, there is a high risk of the infection spreading within the herd. Specific information and advisory services are given to the farmers as how BLV-infection can be prevented:

Separate newborn calves immediately from infected mothers
Buy recruitment only from free declared herds
Isolate known infected animals until slaughtering
Slaughter of all infected animals as soon as possible
In infected herds, give newborn calves colostrums but not milk
Only use sterile pliers for ear tagging
Only use sterile instruments or disposable equipment as needles and syringes

3. After slaughtering of all infected animals in a herd, repeated sampling and analysis of all individual animals are carried out. When there are two consecutive batches of negative analyses from all animals older than 12 months with at least 4 months interval, the herd is officially declared free from leucosis. The first sampling must be done more than 4 months after complete slaughtering of all infected animals. Thereafter follow-up checks are made at given intervals.

The EBL programme started 1990 and had gradually been developed into a complete eradication programme which can be adjusted and used for other infection diseases of a similar nature. In Sweden this system is also used as a model for eradication schemes for bovine virus diarrhoea (BVD), Aujeszky´s disease and IBR-IPV.

In the EBL programme, there was found and slaughtered 55,000 infected animals in 6,000 herds and in the IBR-IPV programme the infection was found in 29 herds.

Sweden was officially declared free from IBR-IPV during 1998 and from EBL in January 2001.

For a period of at least five years, Sweden shall take samples for EBL analysis from bulk milk from all milk-producing herds and individual samples from all animals older than two years in 2,300 beef herds. The milk samples are also analysed for IBR-IPV and out of the individual samples for EBL, 3,000 are choosed for analysis for IBR-IPV.

# Clinical manifestations of bovine virus diarrhoea virus (BVDV) in cattle and methods for control

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## Introduction

Bovine virus diarrhoea virus is an enveloped virus belonging to the genus *Pestivirus* in the family *Flaviviridae*. This genus comprises bovine virus diarrhoea virus (BVDV), classical swine fever virus (CSF) and border disease virus (BDV); viruses that are closely antigenically and genetically related. BVDV can cross infect cattle, sheep and pigs and BDV sheep and pigs whereas CSFV seems restricted to pigs. There are two genotypes of BVDV, BVDV type 1 and type 2 and two biotypes, cytopathic and noncytopathic. The cytopathic viruses may arise from noncytopathic viruses through mutation and are most commonly isolated from persistently infected (PI) animals that develop mucosal disease (3,4).

Bovine virus diarrhoea virus (BVDV) infections are prevalent in cattle populations all over the world and causes huge losses directly caused by BVDV and also indirectly because of the immunosuppressive properties of the virus (7,8,10). In countries with an intensive cattle production, BVDV is probably the single most important virus infection causing impaired herd health and production losses (10). Despite the fact that excellent diagnostic techniques have been available and that the epidemiology of BVDV and effective methods to control the disease have been known for more than a decade (1,13,16) only a few countries have schemes in order to control or eradicate this virus from the cattle populations on a regional or national level (13). The reason is probably that most herd owners and veterinarians do not recognize the importance of this virus infection and do not believe it is possible to keep herds free from this infection in cattle dense areas. However, it is possible and many infected herds are cleared from the infection without any intervention at all, besides controlling or stopping the animal trade between herds (13,21).

## Clinical manifestations of BVDV

The different clinical manifestations of BVDV have recently been excellently reviewed by Radostits et al (20) and in this abstract of my presentation only a brief summary is presented.

Most infections with BVDV in non-pregnant animals are subclinical and cause a mild transient disease with fever, mild diarrhoea, transient leucopenia and recovery within a few days. However, severe outbreaks of primary BVDV type 2 infections causing high mortality rates in cattle of all age groups and thrombocytopenia and hemorrhagic disease in veal calves, have been reported from North America (5,6). BVDV type 2 infections are also present in the cattle population in many countries in Europe but have not been associated with such severe disease symptoms, with the exception of an outbreak in the Netherlands caused by a live BHV-1 vaccine contaminated with BVDV type 2. Despite the fact that most BVDV infections are subclinical it seems very clear that these infections are immunosuppressive and may aggravate many other infections (7,8,12,18). This can clearly be seen as an increased general health in herds that becomes free from the infection or as a decreased health status in previously free herds that becomes infected with BVDV (7,12). It also seems clear that BVDV is an important reason to repeat breeding since it has been shown that acute infections

with BVDV before or at the time of breeding clearly diminish the fertility in susceptible cows that becomes infected during the time of insemination (9,11,12,15). In adult bulls the acute infection may be associated with a transient impairment of semen quality and shedding of virus in the semen (11,19). PI bulls always shed large amount of virus in the semen. A localized persistent BVDV infection has also been identified in a seropositive bull (named "Cumulus") at an AI centre (22) and semen of this bull infected one out of three inseminated seronegative heifers (17). The huge reproductive losses caused by BVDV are due to the fact that this virus with 100% efficiency crosses the placenta when it infects a pregnant non-immune animal. Since cows are pregnant most of their lifetime, there is a great risk that they will be infected with BVDV during this time period. This risk of infection during pregnancy can be estimated between 10 to 30% for cows in dairy herds in countries endemic infected with BVDV (10). Depending on the age of the foetus the infection will result in foetal resorption, abortion, mummification, congenital malformation, birth of immunotolerant PI calves or birth of weak undersized calves or calves infected in late pregnancy that seems apparently healthy (20). However it is my experience that also calves that have been infected late in pregnancy and appears normal at birth are immunosuppressed and have a decreased growth rate compared to normal calves.

**Methods for control**

Vaccines against BVDV have been used in many countries for more than 40 years now without any success. No publication has shown any of the vaccines to be convincingly effective in preventing foetal infections in field trials. BVDV vaccines are of two different types; live or inactivated (4). The live vaccines may have an immunosuppressive effect and if PI animals are vaccinated, the vaccine virus can recombine with the virus that these animals carry and also precipitate mucosal disease in the vaccinated animals. In my view it is clearly a risk that this might generate new strains of BVDV, with a possible increased virulence. It is also worth noting that BVDV type 2 strains seems to have a high prevalence only in countries that use a lot of different live virus vaccines (13). The inactivated vaccines on the market are probably safe to use in contrast to the live vaccines. However I cannot see that the inactivated vaccines now in use are any better than the inactivated vaccines produced in the USA for more than 20 years ago. My experience when I tested such a vaccine in 1984 was that it produced a to short and narrow immune response, to be of any significant value in the control of BVDV.

Systematic eradication of BVDV without vaccination started in Scandinavia (Sweden, Denmark, Norway and Finland) in 1993 based on similar principles (1,2,13). The schemes include as a first step identification of non-infected and infected herds using different combination of serological herd tests such as bulk milk tests and sample of animals in a certain age (spot tests). The second step is certification of non-infected herds by repeated sampling. In all countries except from Denmark an indirect commercial ELISA (SVANOVA) is used for the antibody determinations in serum and milk (13). In Denmark an in house blocking ELISA is used. This ELISA seems to be less sensitive regarding analysis of bulk milk compared to the indirect ELISA (unpublished observation). Using the indirect ELISA (SVANOVA) it is possible to identify animals with a high risk of carrying PI foetuses by determination of the antibody levels in late pregnancy (14). This is not possible in the same way by using a blocking ELISA.

In infected herds virus clearance is aimed at removing persistently infected animals in a cost- and time-efficient manner. However it has been observed that a common finding is *self-*

*clearance*, where the infection ceases without any other intervention than stopped or controlled introduction of new animals (13,21).

The Scandinavian countries are now on the way to a total freedom from BVDV. A similar regional programme in Austria has also been very successful in controlling the disease and BVDV has been eradicated from the Shetland Islands. However, in several regions in Europe, systematic control efforts against BVDV appears to be hindered by decision makers, veterinary researchers and practitioners that think vaccination is the only option for BVDV control. This even if the animal health veterinarians in the field and farmers have understood the benefits of cheap and efficient control program against BVDV (1,2,13), without the use of vaccines. The main argument from those who do not want any control programme, which is not based on vaccination, is that eradication of BVDV creates free herds that are very susceptible to new infections. This is true but it is easy to keep them free from BVDV in the future. I hope more countries will start similar systematic control programmes as in Scandinavia, since this will be an important step for a better cattle health worldwide. Maybe the Baltic States will be in the forefront when it comes to controlling or eradicating BVDV without the use of vaccines (21).

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# Epidemiological Studies of BVDV and Herpes Virus Infections in AI Centers in Lithuania

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Bovine viral diarrhoea virus (BVDV) belongs to the *Pestivirus* genus in the *Flaviviridae* family. Noncytopathogenic (ncp) biotype of the virus is characterized by a vertical virus transmission and is responsible for a permanent BVDV circulation in cattle population. The significant economic impact are inflicted due to productive and reproductive losses. Investigations of the causes of BVDV spreading and features of its manifestation and distribution are based not only on the analysis of the agent and its properties, ways of transmission and immune status of the animal, but also on the analysis of various other factors and their importance. The existing differences in the structure of cattle population, keeping and care conditions may cause variable distribution patterns of BVDV infection.
Infectious bovine rhinotracheitis (IBR), also called infectious pustular vulvovaginitis, is an infectious disease caused by the bovine herpes virus type-1 (BHV-1). In young animals this disease manifests itself as an inflammation of the upper respiratory tract or encephalitis, whereas in adult animals the infection either causes no symptoms or manifests itself as balanoposthitis, vulvovaginitis and reproduction disorders. Preliminary serological investigations for IBR made in 1992 showed 52.5% seroprevalence in animals at artificial insemination (AI) and bull-growing centers. At the same time analysis of the relative importance of major diagnosis in cattle during the period 1992- 1993 revealed that up to 30 % and 6 % of cattle suffered from respiratory and genital diseases respectively. Based on these investigations, the Lithuanian infectious bovine rhinotracheitis/pustular vulvovaginitis (IBR/IPV) control programme was developed and approved on 7 October, 1993.

The major goals of the current survey were: (1) to investigate the prevalence and incidence of BHV-1 and BVDV infections in AI centers bulls; (2) to investigate the quality of bull semen obtained from AI centres and semen banks, using virus isolation techniques; (3) to evaluate the efficiency of IBR control programme, (4) to determine the possible influence of different factors on the distribution of BVDV infection.

Blood serum samples from bulls at AI centers and bull-growing stations were examined by an enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to BVDV and BHV-1.Closed one – tube reverse transcription nested polymerase chain reaction (RT – PCR)was used for detection of BVDV. Primary cells from bovine testicles were used for BHV-1 isolation and identification.

The studies in 1997 – 2000 of the prevalence of BVDV infections in six AI centers determined 55.1 % of seropositive animals. The obtained results of investigations revealed different situation in all AI centers. The carriers of antibodies to BVDV consisted from 20.1 till 92.2 % of bulls keeped in different AI centers. Besides, in some AI centers during four years the number of seropositive animals increased.
Animal age is an important factor, which together with environmental factors is responsible for changes of physiological and immune status of an animal, metabolism and productivity. Besides an examination of blood samples for antibodies to BVDV in different age groups of animals provides the information about the infection circulating in a herd. Analysis of age

influence on disease distribution included 439 animals divided into 8 groups. The smallest number of seropositive animals was determined in the age group <1year (19.4 %). In the third and consecutive years of life the number of seropositive animals increases reaching its maximum in group of animals aged 5 years and older (67.8 – 72.7% ). Thus, we may concern that the animal age is in direct correlation with the number of seropositive animals.

The seroprevalence of BVD may be affected by various risk factors. Therefore, it is important to calculate the annual incidence risk of this infection on the basis of a relevant dataset. The annual incidence risk of BVDV infection determined according to specific distribution of antibody carriers depending on the animal age revealed that the younger (aged 1- 3 years) bulls were subject to a greater risk of infection. The annual incidence risk of BVDV infection among them was 0.30, whereas among the older animals groups only half of this value (0.13).

At four large and two smaller Lithuanian AI centers, blood serum analysis revealed that before the implementation of the IBR control programme 49.8% of bulls were infected with BHV-1. Therefore, the IBR eradication programme was most strictly implemented on those farms and as result only 3.5% of the bulls tested appeared seropositive in 1996. All these bulls were culled and no seropositive bull was detected since 1998.

Bull semen samples were also examined for BHV-1 contamination. In 1993 semen samples from 65 seropositive bulls from AI centers were examined and in 16.9% of the cases BHV-1 was isolated and identified in cell culture after 3-4 passages. However, it was observed that the bulls that were examined had recently suffered from balanoposthitis. Semen stored in liquid nitrogen was also examined. In 1994 and 1995, 5.3% and 2.8% of these samples were diagnosed positive for BHV-1, respectively. The BHV-1 contaminated semen lots were eliminated.

The epidemiological features of BVDV vary from country to country and may differ considerably. This has been proved also by our investigation data. Analysis of the animal age influence to the BVDV infection spreading in AI centers was determined the trend, which showed that with age of animals increase the number of seropositive animals. In our opinion the dynamics of seropositive animals in different age groups is regular in character.

The incidence of infection showed a tendency to lower risk among older animals compared to younger animals in Lithuania. Our data differ from the data obtained by Danish scientists (Houe et al., 1995), who determined that in Danish dairy herds the annual incidence risk of BVDV infection was higher among the older animals simultaneously in Michigan (USA) this trend was not determined. It should be pointed out that the herds examinated in Denmark and USA included no PI animals. Moreover, previous investigations (Houe H., Meyling A., 1991) in random dairy herds revealed that the annual incidence risk of BVDV infection were approximately similar in all age groups. The different values of infection annual incidence risk and its trends in different animal age groups of various countries may be accounted for by dissimilarities in herd size and structure, animal keeping conditions and mobility, as well as by a different number of PI animals in herds.

Serological testing of bulls from AI centers carried out in 1994 and 1995 revealed that the initial implementation of the IBR/IPV eradication programme in Lithuania was effective. The number of BHV-1 seropositive bulls significantly decreased from 49.8% in 1993 to 3.5% in 1996 and no seropositive bull was detected since 1998. However, investigations carried out in 2001 revealed that 8.4 % of cattle on different dairy farms have antibodies to BHV-1 virus.

Therefore these may be considered virus carriers and remain a source of reinfection. Furthermore, the results of our survey could be indicated with occurrence of reproductive disorders in Lithuanian cows.

# Health surveillance programs with special reference to infectious diseases in Estonia

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## Introduction

The systematic health surveillance programs on farm animal infectious diseases are conducted almost entirely by the government. The only exception is the udder health surveillance program, which is conducted by the Milk Analysis Laboratory of Animal Recording Centre (a government body) and paid by the farmers.

The government financed surveillance program of farm animal infectious diseases is run by Veterinary and food Board and the Veterinary and Food Laboratory.

Since 1999 the legal bases for the program is the Animal Disease Act. The program annually determines the diagnostic investigations and the numbers of tests performed for surveillance of diseases controlled by the state. The program determines also the annual vaccination schemes paid by the state and the scheme of farm inspections.

The necessary samples are collected and the authorized veterinarians perform the tuberculin tests. The authorized veterinarians are private practitioners who are contracted by the Veterinary and Food Board to perform the necessary field work in the framework of the surveillance program.

## The program for 2002

The program of 2002 has only minor changes compared to the programs conducted during last three years. The following diseases were included to the government farm animal disease control program in 2002:

### 1. Bovine animals

**1.1 Bovine tuberculosis –** tuberculin testing (number of tests: 185850)
All milking cows, heifers and bulls used for insemination are tested once a year with intradermal tuberculin test. All bulls in the artificial insemination (AI) centres are tested twice a year.
Animals giving positive result in tuberculin test are slaughtered and samples for bacteriological investigation are taken.

**1.2 Bovine brucellosis**
Serological investigations – one third of herds are tested annually from pooled milk samples. Number of animals included to the investigation is approximately 42000
All bulls in AI centres are examined once a year.
Aborted fetuses are investigated bacteriologically.

**1.3 Enzootic bovine leukosis**
Serological investigations (total number of tests: 125 000 – in Bovine Leukosis Virus (BLV) free herds milking cows are examined once a year from pooled milk samples. In BLV positive herds (the number in 2001– 12) the testing is conducted according to eradication program.
All bulls in AI centres are tested twice a year.

**1.4. Bovine paratuberculosis**
Serological examination of cattle older than four years in a sample of herds (number of tested animals 1750).
All bulls in AI centres are tested serologically once a year.
A sample of seropositive animals investigated bacteriologically from fecal samples.

**1.5. Bovine spongiform encephalopathy**

Detection of agent in brain samples with rapid test (Platelia ELISA) – animals over age of 24 months died on farms, sent to emergency slaughter, found sick at normal slaughter or imported from countries were BSE has occurred after importation (number of tests 1150).
Histological investigation of brain of animals clinically suspected of having BSE or positive in rapid test.

**1.6. Investigations specific to AI bulls**
**1) Leptospirosis** – serological examination annually with micro-agglutination test;
**2) Trichomonosis –** isolation from semen twice a year;
**3) Bovine campylobacteriosis –** isolation from semen twice a year;
**4) Chlamydiosis –** serological examination once a year;
**5) Bovine viral diarrhoea –** virus detection once a year;
**6) Infectious bovine rhinotracheitis –** virus isolation from semen.

**1.7 Salmonellosis –** bacteriological investigation of fecal samples (700 tests)

**2. Porcine animals**

**2.1. Tuberculosis ( number of tests : 68400)**
Intradermal tuberculin testing of pigs from parent stock of breeding herds.
All boars in AI centres are tested once a year.
Animals giving positive result in tuberculin test are slaughtered and samples for bacteriological investigation are taken.

**2.2. Serological surveillance of breeding pigs:**
Porcine brucellosis, leptospirosis, classical swine fever, swine vesicular disease, Aujeszky disease, transmissible gastroenteritis, porcine reproductive and respiratory syndrome and atrophic rhinitis of pigs

A sample of pigs from parent stock of breeding herds and all boars in AI centres and other boars used in insemination are tested once a year. Number of tested pigs: 1500

**2.3 Serological surveillance of slaughter pigs**
Leptospirosis, classical swine fever, swine vesicular disease, Aujeszky disease, transmissible gastroenteritis, porcine reproductive and respiratory syndrome.

A sample of slaughter pigs are tested once a year. Number of tested 4000

**2.4 Salmonellosis –** bacteriological investigation of fecal samples (500 tests)

**3. Ovine and caprine animals**

**3.1. Ovine epididymitis**
Serological examination of rams of parent stock once a year. Number of tests– 112
**3.2. Ovine brucellosis**
Serological examination of parent stock of breeding herds serologically once a year. **N**umber of tests – 1537.
**3.3. Scrapie**
Detection of agent in brain samples with rapid test (Platelia ELISA) from animals which died on farm or were slaughtered and had neurological symptoms. Number of tests – 30.
Histological investigation of brain of animals clinically suspected of having Scrapie or positive in rapid test.
**3.4. Maedi-Visna**
Serological examination of a sample of parent stock of breeding herds once a year. Number of tests – 2345

**4. Horses**

**Serological examination of stallions and mares used for breeding before the mating season**: Equine infectious anemia, Glanders and Dourine. Number of tested animals was 1020.
Stallions and mares used for breeding serologically before the mating season.

**5. Poultry**

**5.1. Avian tuberculosis**
Tuberculin testing of 10% of the parent stock of breeding herds annually. Number of tests – 700.

Birds giving positive result in tuberculin test are slaughtered and samples for bacteriological investigation are taken.

**5.2. Salmonellosis**

1) Bacteriological investigation of fecal samples from sample of birds (number of tested poultry– 16960).

2) Blood agglutination test to Pullorosis of a sample of birds in a herd (number of tests: 6700).

**5.3. Newcastle disease(number of tests: 3450)**
Serological examination of 0,5 % of parent stock from laying hen herds and breeding herds.

## Conclusions

As it appears from previous, the government disease surveillance program is first of all orientated to the control of exotic diseases for Estonia (e.g. OIE list A diseases and several list B idseases) and also to so called politically important diseases like tuberculaosis, brucellosis and bovine enzootic leukosis. Concerning the diseases present in Estonia, the priority is laid on surveillance in breeding herds (animals) and AI centres.

# AI situation in Lithuania

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In Lithuania, first Artificial Insemination (AI) centres have been established in 1956 and since then majority of Lithuanian bovine stud has been artificially inseminated. Since 1975 only deep-frozen bull semen is used for AI. Semen doses were accumulated in state-established AI centres and for that period of time, from 90 to 95 percent of all cows and heifers were artificially inseminated.

For the present moment, there are 3 AI centres in Lithuania (were 5 till the beginning of 2002) that collect, process and distribute bull semen. These centres recruit bulls from a bull-rearing centre. In total, AI centres have 90 bulls that are being collected and their semen is used for AI. Still few doses of semen from high genetic merit sires is also imported from abroad, and to-day AI centres in Lithuania have 5.4 million semen doses accumulated in their reservoirs.

Following the reorganization Lithuanian agriculture sector in 1991, private artificial insemination organizations have been established. The activities of these centres are licensed for insemination of cows and heifers that are owned by agricultural cooperatives and private farmers. The State animal breeding supervision service under the Ministry of Agriculture issues licences for semen production, distribution and artificial insemination activities. And the AI is organized according to the cattle improvement programmes. The genetic value of bulls is estimated according to BLUP methodology. All information concerning AI, productivity control of cattle is accumulated in database of Public institution Rural Business Development and Information Centre, where it is being further processed and analysed.

According to the official records (2002.11.01.) there are 931.187 heads of cattle in Lithuania, of which 491.690 are cows, and 50.000 of breeding age heifers. According to the prognosis, approximate AI figures throughout the year 2002 are 4000.000, that is approximately 74% of total cow/heifer population in Lithuania.

According to the Lithuanian Animal Tagging Registry, out of 931.187 registered animals, 642.087 animals are of Black and White breed, 252.523 animals are of Red and White breed, 4.189 are of other breeds, 1.914 are of beef breeds and 30.474 are not purebred (mixed breed). Only 135.397 cows, owned by agricultural co-operatives and farmers, i.e. 29% of all cow population are enrolled in milk recording system. Of these, 98.682 cows are of Black and White breed, (of them: 95.834 Lithuanian; 1.223 Holstein; 1.209 German; 216 Danish; 83 British Friesians; 68 Swedish and 49 Dutch), 36.355 cows are of Red and White breed (of them: 34.799 Lithuanian; 435 Airshire; 350 Swedish; 271 German; 230 Angler; 205 Danish; 40 Fleckvieh and 20 Holstein. Of local breeds, 154 cows of improved Whiteback and 195 cows of Lithuanian Grey Cattle are enrolled in milk recording system. In all controlled herds there are 98.000 heifers of various dairy breeds.

Insemination index in controlled herds is -1.9 for cows, and 1.6 for heifers. Semen, produced in Lithuania is used for insemination at the largest scale. Still, semen from Canada, USA, Denmark, Sweden and Germany has been imported. In year 2001, and 2002, - 15.489 and 198.410 bull semen doses have been imported to Lithuania respectively.

In Lithuania, 490 persons possess licenses for work in field AI, and are registered in a database. Each Insemination is registered in an insemination certificate, and all data concerning the insemination is then recorded in a central database of Public institution Rural Business Development and Information Centre, where it is being further processed and analysed.

Problems of AI work in Lithuania are mostly associated with overall situation of agriculture in Lithuania, such as Governmental policy of agriculture as well as financial economical and social situation in agricultural sector.

Boar semen production units

There are 8 boar semen production units in Lithuania that annually produce 110.000 insemination doses. The fertility results after AI in pigs vary from 60 to 91%. Over the recent years, pig production industry has been a subject of major changes – with the change in ownership in pig production units, there is a tendency of these units to become larger, more centralized. This means that modern technologies are being applied in this sphere, which also implies new technologies of pig breeding and insemination. Small farmers rear still rather many pigs under primitive conditions. Insemination is also used in this sector, but at a very low scale.

Artificial insemination in horses

For the present moment, there is one private owned insemination centre of mares in Lithuania, and there are no official figures over AI in horses. Here a lot of work still needs to be done, but all activities are governed by local market of horses, which is at its starting point.

During the latest 10 years the following animal breeders organizations have been established:

1. Lithuanian Black and White cattle breeders association;
2. Lithuanian Red and White cattle breeders association;
3. Holstein cattle breeders association;
4. Lithuanian beef cattle breeders association;
5. Lithuanian pig breeders association;
6. Lithuanian horse breeders association;
7. Žemaitukai horse breeders association;
8. Lithuanian Trakenai horse breeders association;
9. Baltic Hanoverian horse breeders association;
10. Race horse league;
11. Lithuanian sheep breeders association.

A governmental body - State animal breeding supervision service under the Ministry of Agriculture, supervises all animal-breeding activities:
It has the following tasks:

1. Implements state's policy in animal breeding sector, controls implementation of legislation associated with animal breeding activities,
2. Distributes funds allocated for animal breeding sector, and controls their utilization;
3. Controls implementation of animal breeding programmes,
4. Issues licenses (for private and legal bodies) on animal breeding activities,

5. Issues certificates for import (export) of breeding material,
6. Takes part in selection and evaluation procedures of animal breeding studs and farms,
7. Prepares drafts of legal act concerning animal breeding issues and subjects for Ministry of Agriculture,
8. Subjects proposals to the Ministry of Agriculture concerning development of animal breeding work,
9. Controls utilization of means subsidizing animal breeding,
10. Scrutinizes declarations, complaints and suggestions of private and legal bodies concerning the quality of breeding material, recording and reliability of data,
11. Controls accumulation and storing of data on animal breeding,
12. Certifies forms of records in animal breeding,
13. Controls registration of animals in herd books and issuing of rolls and catalogues and all other publications associated with animal breeding,
14. Controls evaluation of sires according to their individual features and progeny productivity,
15. Certifies sire selection plans of officially recognized animal breeding centres and institutions and controls their implementation,
16. Attests certificates of origin or assigns this work for officially recognized animal breeding institutions,
17. Controls purchasing and selling of pedigree animals,

**Reproductive technologies and semen quality evaluation:**

In total, in Lithuania we have over 5.4 million bull semen doses stored in a liquid Nitrogen tanks. Of this, 37.5 thousand semen doses are in National Gene bank project and are of national cattle breeds – 14.7 thousand straws of White back breed cattle, and – 22.8 thousand straws of Grey cattle breed. No ova or embryos of these breeds are stored in gene bank. No local pig breed semen is stored in this gene bank.

In Lithuania, before being used in field AI, bull semen needs to meet certain qualitative requirements, defined as “Bull semen, quality requirements”. The quality requirements consist of two parts – one dealing over fresh (neat), and the second – over frozen – thawed semen. Briefly, neat semen quality requirements define the semen that can be further processed and frozen in individual insemination doses, and quality requirements of cryopreserved semen define the criteria cryopreserved semen must meet after thawing in order to be used for insemination.

Animal reproduction laboratory, of Lithuanian Veterinary Academy routinely screens semen samples that are send in by bull/boar stations, private animal keepers. Annually, over 800 semen samples are screened for sperm morphological abnormalities, motility, and sperm viability. Bull and boar stations are suggested to screen sire semen quality on quarterly basis, however most samples evaluated are samples of problem cases, or emergency samples, mostly due to decreased conception rates in the herds.

Boar semen samples comprise more than 80 % of all semen samples evaluated and majority (86,5%) of boar semen samples are provided by private persons (from animals kept at their herds). Bull semen samples are only provided by bull stations. This year, the average percent value of morphologically abnormal spermatozoa, was 21.36∂17.51 %, (ranged from 2% to 95.4%). The most common pathology was proximal and distal cytoplasmic droplets, which incidence accounted to 5.11 ∂ 9.39% and to 5.7 ∂ 7.12% respectively.

Concerning bull semen, the average percent value of morphologically abnormal spermatozoa was 14.32 ∂15.48%, and ranged from 2.5% to 48%. Head abnormalities were the most common sperm abnormalities seen, that accounted to 6.33∂0.28% ranges from 0.8% to 13%). The second common pathology was pathological heads which incidence accounted to 4.03 ∂ 1.06%, and ranged from 0% to 19.5%.

According to officially accepted (Standardization Department) sperm quality requirements, semen sample cannot be used for insemination if pathological spermatozoa of this semen sample exceeds 25 %, and of these are more than 15% with pathological heads, and 20% with tail pathologies.

# The use of A.I. in cattle and horses in Estonia

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## A.I. in Cattle

The beginning of herdbook keeping can be considered as a start of the directed breeding of agricultural animals. There has been a continous development from the herdbook in 1885 to the practice of biotechnological method with the aim to use the valuable breeding material more efficiently. Breeding success without artificial insemination nowadays is impossible.
The first data about artificial insemination in cattle in Estonia belong to 1938 when Edgar Keevallik made an experiment in Kuusiku inseminating cows with fresh semen straight after collection from bulls.

*AI stations and bulls*

There were 107 bull stations with 310 bulls in Estonia in 1935. In 2003 we have only one AI station with 115 (90 Estonian Holstein and 23 Estonian Red) bulls and it is located in Kehtna. This station belongs to Animal Breeders´ Association of Estonia (ABAE) which in addition to semen collection, handling and marketing deals with breeding and feeding advising and herdbook keeping. There are 20-25 Holstein and 10 Estonian Red bulls tested per year. According to that fact the semen collection is devided into three periods.
In the first period, 1500 doses of semen from a young bull are collected for test inseminations. During that period semen quality is being checked very thoroughly and after that it will be decided if the bull will be suitable for breeding.
During the second period, 10000-15000 doses of semen will be collected and according to the exterior, origin and pedigree of a bull a decision about the use of the bull in breeding is made.
On the third period as many doses as possible will be collected from the proven bulls.
During the first and the second periods, semen is diluted after collections so that there will be 30-40 x $10^6$ spermatozoa per straw and on the third period, 18-20 x $10^6$ spermatozoa per straw.

*Handling of semen and quality control*

In Kehtna AI station semen is packed into 0,25 ml French straws. The semen is diluted with a commercial extender Triladyl™ (Minitüb, Germany).
The procentage of pathological spermatozoa is calculated from each ejaculate and sperm motility is estimated by CMA (Computer Assisted Sperm Motion Analysis, Minitüb, Germany). The following motility characteristics are estimated: motility, progressive motility, velocity curve line, velocity average path, velocity straight line, linearity, beat cross frequency and amplitude of lateral head displacement.
Fresh semen is eliminated if the percent of pathological sperms is >20 and the percent of progressively motile sperms is below 75.
The dilution of semen is made according to sperm concentration (which is measured by colormeter SDM-5) and semen motility characteristics. After freezing the semen motility is tested by CMA, estimating the same characteristics as in the fresh semen. The frozen semen is eliminated if the progressive motility of semen is below 50%. To get better outline of semen quality intactness of sperm membranes is tested by hypoosmotic test (HOT).

*Storage and marketing of semen*

After 30 days in quarantine the semen doses are sent to the main storage. Before distribution of semen to the farmers the motility of semen is tested under the microscope and the semen will be eliminated if the progressive motility of semen is below 40%.

The semen are distributed to the AI technicians according to their orders in the regional distribution locuses and it is taken there by our company transport. The distribution is on the certain days of the week and therefore the orders must be done at least three days in advance. All farmers can order also the world top bulls. In 2002 ABAE imported 20000 doses of semen and exported 1000 doses of semen (mostly to Latvia).

**Trainig of AI technicians**

ABAE employs 14 AI technicians, other certified technicians have direct contracts with farmers. The training of AI technicians is done by ABAE and the Estonian Agricultural University. The licence is issued by ABAE. AI technician has to participate in the training courses every year to get the licence or to extend it. Altogether 300 AI technicians have got licences from ABAE. The cost for a single insemination is about 10-15 EUROs and 1/3 of it constitutes semen.

**Reports of AI and pregnancy**

Information about bovine insemination and pregnancy is registered and published by independent organization Animal Recording Centre. The results of inseminations and pregnancy are forwarded to ABAE twice per year (Table 1). ABAE gets such reports for bulls, herds and AI technicians. The NRR by years and breeds is given in Table 2.

Table 1 Artificial insemination and non-return rate in 2001.

| | ER | | | EHF | | | EN | | | All breeds | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cows | Heifers | Total | Cows | Heifers | Total | Cows | Heifers | Total | Cows | Heifers | Total |
| No. of females inseminated | 21709 | 6527 | 28236 | 69027 | 20217 | 89244 | 351 | 133 | 484 | 97357 | 27992 | 125249 |
| No. in milk recording herds | 20141 | 6299 | 26440 | 66508 | 20013 | 86521 | 274 | 123 | 397 | 91634 | 27574 | 119208 |
| NRR 90 d.% | 55,3 | 68,1 | 58,4 | 51,6 | 67,7 | 55,4 | 72,5 | 72,6 | 72,5 | 53 | 68,2 | 56,6 |
| No. of inseminations in milk recording herds | 34489 | 9149 | 43638 | 122840 | 29050 | 151890 | 357 | 155 | 512 | 163983 | 39688 | 203671 |
| No. of inseminations per cow | 1,8 | 1,5 | 1,8 | 1,9 | 1,5 | 1,8 | 1,4 | 1,3 | 1,4 | 1,9 | 1,5 | 1,8 |

ER-Estonian Red
EHF- Estonian Holstein
EN- Estonian Native

Table 2. 90 days-NRR by years and breeds.

| | 1998 | 1999 | 2000 | 2001 | 2002* |
|---|---|---|---|---|---|
| Estonian Red | 64,7 | 64,8 | 66,1 | 58,4 | 68,9 |
| Estonian Holstein | 58,6 | 59,7 | 60,0 | 55,6 | 66,6 |
| **Estonian Native** | - | - | - | 72,5 | 78,6 |

* 01.01.2002-30.06.2002

**A.I. in Horses**

The total horse population in Estonia is approximately 6000. This number has start to increase compared to year 1998, when there were approximately 4500 horses in Estonia. The biggest horse population was in Estonia in 1939 when more than 220 000 horses were counted.

In Estonia, there are at the moment 4 main horse breeds: Estonian Horse, Tori Horse, Estonian Draught Horse and Estonian Sporthorse (Trakhener, Hannoverian, Holsteiner etc.). There are two breeding associations. Breeding of Estonian Horse, Tori Horse and Estonian Draught Horse is co-ordinated by Estonian Horsebreeders Society and Estonian Sporthorse breeding is co-ordinated by Estonian Sporthorse Breeders´ Society which was established 26.04.2000.

There is a private company Gynaecology Centre "Saare Tallid" where most of A.I. procedures in horses are made in Estonia (Table 3.). Estonian Sporthorses are main clients in this centre.

Table 3.Use of A.I. in Gynaecology Centre "Saare Tallid" 1999-2002.

| Year | No of mares | With frozen semen | With fresh semen | No of stallions |
|---|---|---|---|---|
| 1999 | 53 | 19 | 34 | 17 |
| 2000 | 96 | 29 | 67 | 21 |
| 2001 | 101 | 24 | 77 | 26 |
| 2002 | 114 | 29 | 85 | 28 |
| Total | 364 | 101 | 263 | |

Estonian Horse, Tori Horse and Estonian Draught Horse breeders use natural mating. Data of borned foals and used stallions are presented in Table 4.

Table 4. Number of foals and used stallions 1999-2001.

| Year | 1999 | | 2000 | | 2001 | |
|---|---|---|---|---|---|---|
| Breed | No foals | No stallions | No foals | No stallions | No foals | No stallions |
| Tori | 94 | 32 | 103 | 36 | 102 | 38 |
| Estonian | 82 | 27 | 105 | 24 | 86 | 20 |
| Estonian Draught Horse | 14 | 5 | 14 | 3 | 8 | 3 |

# On the detection of the virus infections in calves in Estonia

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## Introduction

The problem of respiratory and enteric virus infections among calves became serious after the concentration of cattle into large farms. Economic losses increased as a result of mistakes in the management. The aim of the studies was to determine the distribution of virus diseases of calves, their nosological structure and the epidemiological situation.

## Materials and methods

The research was carried out from 1984 to 2002.
For virological studies, ocular and nasal secretions from live animals and internal organs of dead or emergency slaughtered animals, as well as and faeces samples were taken. For the detection of virus-specific antibodies, serum samples were screened.

For the isolation of virus strains, the monolayer of the primary cell culture of the foetal calf``s kidney and calf testicles, the permanent cell line MDBK (Madin Darby bovine kidney) and BK-80 (bovine kidney) were inoculated with a 10% suspension of organs in Eagle MEM (Minimum Essential Medium). The virus antigens were detected by immunofluorescence assay, passive haemagglutination test and immunodiffusion reaction. The isolated virus strains were identified using serumneutralisation test and ELISA.

Serum samples for the virus-specific antibodies were investigated by virus neutralisation test, passive haemagglutination assay, ELISA and haemagglutination inhibiton test.

In all the pathological materials based on 1705 calf was examined virologically. The total number of serologically tested serum samples was 8358 .

Calves were investigated to bovine herpesvirus-1 infection (BHV-1), bovine adenovirus infection (BAV), bovine respiratory syncytial infection (BRSV), bovine virus diarrhoea infection (BVDV), parainfluenza-3 (PI-3) and bovine rotavirus infection (BRV).

The results of the detection of respiratory and enteric viruses of calves and determine in the virus-specific antibodies during 18 -year investigation period presented in Fig. 1 and 2.

## Results and discussion

For the detection of bovine infectious rhinotracheitis (IBR), 1866 samples from the calves were examined, and the virus was found 128 cases (6.8%). BHV- 1 was sporadically diagnosed among calves from the beginning of 1986 and simultaneously was
the outbreak of IBR/IPV in the artificial insemination staion. A new outbreak took place in 1991. Since 1993 the IBR infection was diagnosed every year.

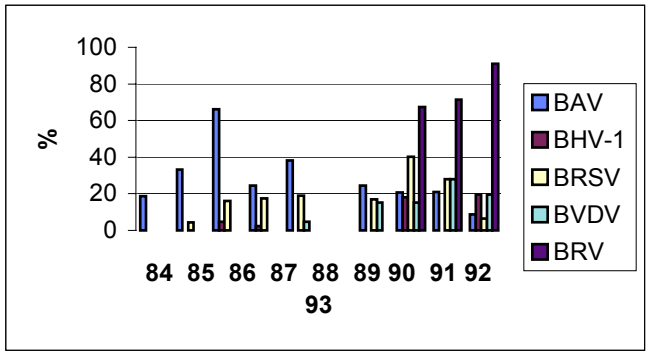


Fig. 1. Results of virological investigations of calves in 1984–1993.

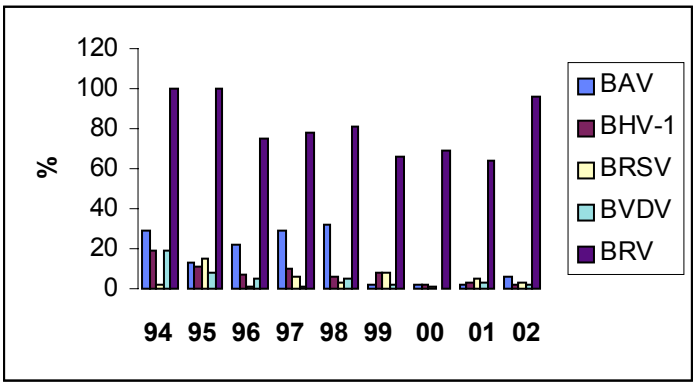


Fig.2. Results of virological investigations of calves in 1994–2002.

Serolgically were tested of 1033 serum samples from the calves, and BHV-1 –specific antibodies were found in 197 cases (19%).
In the same period, 933 serum samples from the cows were investigated, and the numbers of positive results were 209 (22.4%).
From the 1866 samples were collected for finding bovine adenovirus, results were positive in 391 cases (21%). The evidence of adenovirus infection was variable, the presence of BAV infection showed a significant increase in 1985, 1991, 1997-1998. In addition, the frequency of the occurrence of I and II subgroup was ascertained in 328 cases. The results demonstrate that the presence of both subgroups of BAV was identified, namely subgroup I (61.5%) and subgroup II (52.1%). The results of the serological study indicate the intensive spread of the virus, as 36.5% of the investigated calves and 52.7% of the cows were positive to the adenovirus infection.
Bovine respiratory syncytial virus was found 12% of cases. BRSV was first diagnosed in Estonia in 1985. 1986–1990 was a relatively stable period in the distribution of the epidemic, but an increasing occurrence of BRSV infection was noted in 1991, after which that the number of diseased calves decreased again.
Bovine virus diarrhoea was diagnosed in 6.75% of investigated calves. Between 1990-1994 the prevalence of the infection was relatively high, extending from 15.1-27.9%, although in 1995 the persentage of infected calves decreased, but in 2001 the percentage of infected calves increased again.
The epidemiological situation of the parainfluenza -3 was screened serologically. The results confirmed the high persentage of infected calves (61.1%) and cows (56.8%).

For the diagnosis of the bovine rotavirus infection in calves, the faeces samples were tested from 1991, and the virus antigen was found in 77,6 % of the calves.
During the first period (1984-1990) of investigation, the prevaling infection was BAV, but on the second half (beginning in 1991) it was replaced by BRSV, BHV-1 and BVDV.

The average frequencies of detection of mono- and multiple virus infections were compared (Table 1.)

Table1. Virologically diagnosed mono-and mixed infections

| VIRUS | MONOINFECTION % | MULTIPLE INFECTION % |
|---|---|---|
| BAV | 9.6 | 17.9 |
| BHV-1 | 1.2 | 7.5 |
| BRSV | 1.0 | 10.8 |
| BVDV | 1.3 | 4.3 |
| BRV | 8.1 | 67.8 |

The most frequent were BAV and BRSV infections together with other viruses. Comparing mono- and mixed virus infection average frequencies of detection, one may conclude that mixed virusinfections occurred 3.75 times more often. Comparing our results with the work of other authors, we found that Läuchli *et al*. (1990) diagnosed mono- and mixed virus infections in the relation 1:4.
We diagnosed 14 different virus combinations, the most frequent were simultaneously BAV+BRSV, BHV-1+BRSV, BAV+BRV. Three different viruses were diagnosed as following: BAV+BRSV+BRV, BHV-1+BVDV+BRV, BHV-1+BAV+BRSV. The simultaneous occurrence of three or more viruses is also widespread in other European countries (Scott,1994, Wittkowski *et al*., 1994, Murphy *et al*., 1999).
The dependence of the calves` morbidity on age was examined separately. Calves were divided into groups according to age: calves up to one month and more than one month (Table 2). In addition the study had the following research periods: morbidity during spring-summer (from April to September) and fall-winter (from October to March).

Table 2. Dependence of the detection of virus infections in calves in connection with the season and age
October-March

| AGE | NO OF CALVES | BHV-1 % | BAV % | BRSV % | BVDV % | BRV % |
|---|---|---|---|---|---|---|
| < 1 month | 278 | 18.3 | 45 | 17.6 | 12.9 | 79.2 |
| >1 month | 345 | 9 | 14.2 | 8.1 | 9 | 80.5 |
| Total: | 623 | 13.2 | 27.9 | 12.4 | 10.7 | 79.7 |

April- September

| AGE | NO OF CALVES | BHV-1% | BAV % | BRSV % | BVDV % | BRV % |
|---|---|---|---|---|---|---|
| < 1 month | 527 | 3 | 18.6 | 13.5 | 3.6 | 80.5 |
| > 1 month | 142 | 4.9 | 46.5 | 0.4 | 7.7 | - |
| Total: | 669 | 3.4 | 24.4 | 11.4 | 4.5 | 76.9 |

The results showed that BAV, BHV-1, BRSV and BVDV were diagnosed more often in the up to one month age group in the fall-winter period. Rotavirus infection was detected more frequently in the fall-winter time in groups of both age, but the virus did not have a notable role among older calves in the spring-summer period.

Comparing our results with other authors, it appeared that rotavirus was one of the most important pathogen in diarrhoeic calves in Estonia.
In England the rotavirus was detected in 50% of calves, the other enteropathogens were detected at low prevalence (Sondgrass et al., 1988). BRSV and BRV were isolated more often among calves of up to one month of age in the spring-summer season, but in the same period there were the highest incidence of BAV, BHV-1 and BVDV infection in the elder group of calves. The same was stated by Poel et al. (1994). They discovered BRSV more often in autumn and winter.
The data presented here suggest that respiratory and enteric virus infections are widespread in the Estonian calves population and have not yet attained epidemic stability.

Acknowledgements

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# Prevalence of viruses associated with reproductive failure in Lithuanian swine herds

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Swine herds experience the huge losses because of reproductive failure (stillbirths, mummified fetuses, abortion, early embryonic death) due to viral diseases. The main viral pathogenes influencing swine reproduction are porcine reproductive and respiratory syndrome (PRRS) virus, classical swine fever (CSF) virus, Aujeszky's disease (AD) virus, porcine parvovirus (PPV) and transmissible gastroenteritis (TGE) virus. Therefore, the study of epizootic situation have been performed for classical swine fever, Aujeszky's disease, porcine reproductive and respiratory syndrome, porcine parvovirus infection and transmissible gastroenteritis in Lithuanian swine herds which had experienced the cases of reproductive failure in 2001.
For the serological and virological studies the samples of blood sera and pathological material were taken in 2001-2002. The sera samples were tested due to:
ELISA (IDEXX, USA; Test - Line, Czech Republic) with respect to AD
ELISA (NARVAK, Russia with respect to CSF
ELISA (IDEXX, USA; Test - Line, Czech Republic) with respect to PRRS and RT-PCR using primers to european and american strains of PRRSV.
Haemagglutination inhibition reaction (Bioveta, Czech Republic) with respect PPV infection.

Also the pathological material was tested due to:
RT-PCR with respect to PRRSV
Virus isolation and haemagglutination reaction with respect to PPV.

Our study show that Lithuania is free from CSF and AD. All tested sera samples were negative for CSF virus and AD virus antibodies.
The seroprevalence of PRRSV was significantly higher (61.6% ) in 1997 when PRRS disease first recognized in Lithuania and late in 1998-2001 the number of seropositive pigs have shown 38.7%, 40.7%, 36.1%, 29.2%, respectively. Furthermore, our studies indicated that the prevalence of PRRS virus antibody in the Lithuania swine population tested was relatively high (42.2 %) and swine farms with seropositive pigs were widely distributed in the 19 of 23 Lithuanian regions. The seroprevalence studies on PRRSV infection in 16 breeding farms have shown that the number of seropositive pigs in 6 farms ranged from 50.4 to 96.2 %, and only two farms remained serologically negative. RNR of european strain of PRRSV was also isolated using RT-PCR from blood serum samples of sows which experienced the outbreaks of reproductive failure. RNR of american strain of PRRSV was detected only in 4 blood sera samples of pigs from the farms which previously vaccinated pigs with vaccine containing american strain of PRRSV.
TGE was found only in some farms, however the clinical outbreaks were not observed.
With respect to PPV all swine herds were positive. However differences between number of seropositive pigs were found in gilts. It was found that 37.5 % 4-5 months aged gilts were seronegative, 7.5 % ones had passive immunity and 55.0 % ones active immunity. In groups 6 months and 7 months of age 9.3 % and 6.0 % were seronegative, 4.7 % and 16.0 % had passive immunity and 86.0 % and 78.0 % gilts and 100.0 % –active one. 100 % of pigs in groups 8-9 months aged gilts and sows were found seropossitive. Using virus isolation on primary swine kidney cells no virus was isolated from pathological material (samples of lungs and liver of mummified and stillbirths).

# Clinical Results of Using New Immunemodulator in Veterinary Practice in Case of Oncological Diseases

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At the moment there is observed signicficant growth of oncopathology among different species of animals. According to the latest 3-year statistics of veterinary clinic "KAVET", there was observed growth of breast, liver, pancreatic, bones and joint malignant and benign tumours and malignant skin diseases in dogs. Therefore creating new anti-tumour products is the most important medical-biological problem, which, however, does not receive proper progress.

It is known that oncological diseases develop in connection with lowering of functions of immune system. Besides, there react either specific or non-specific factors of immune protection of organism, which causes concomitant complications as viral-bacteriologicallly infection, which causes early death of diseased animal.

In common with staff of P.Stradina Medical Academy of Latvia we have developed, obtained and licenced a new product "Rimolan" for veterinary practice. The product has immunomodulating effect. "Rimolan" is obtained from biologically active tissues of live origin. During studying the product's composition there was discovered that it contains immunologically active albumens which have anti-tumour and anti-bacterial-viral activity.

In pre-clinical experimental studies there was proved that "Rimolan" is non-toxic, non-teratogenic, demonstrates a wide spectrum of immunotrope activity and has reliable effect of inhibition of tumour growth either in case of treating or prophylactic variant.

Clinical approbation of "Rimolan" was done on 15 dogs with oncological diseases of different localization.

The control group consisted of 10 animals, which obtained therapy, accepted in veterinary practice in case of this pathology.

The dose of the product and the scheme of its injecting was worked out considering the main diagnosis, weight of an animal and showings of its immunogram.

Using "Rimolan" there can be achieved dynamics of showing of immunogram (growth till physiological levels of absolute amount of lymphocytes, showings of phagocytal activity of neutrophils, circulating immune complexes and immunoglobulins).

With help of the product the quality of life of a diseased animal can be rised and its life can be elongated.

# Influence of some factors on semen quality of different breeds of boars

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The quality of pedigree material takes important place in pig reproduction. Semen used for AI has to be of good quality, of high fertilizing capacity and of high genetic value. Semen quality depends on multiple biological and environmental factors. Some effects, such as insufficient feeding, high ambient temperatures and aging of the animal have negative effects on sperm production. On the other hand, extended photoperiod, frequent of semen collection and some genetic factors stimulate positively sperm production. Therefore the aim of our study was: to evaluate the influence of semen quality and quantity of Danish Landrace and Duroc boars. The goal for our study was to evaluate the influence of biological and environmental factors on boar semen quality.

This study was performed in Joined stock company "Lek i iai" in 2001. In total, 13 boars of Danish Landrace and 3 boars of Duroc breed were included into the analysis. Ejaculates were collected 3 times during the 2-week period, and assessed for the volume of the ejaculate (ml) and motility (%). Concentration of spermatozoa (billion/ml) and sperm morphology was assessed in Animal Reproduction laboratory in Lithuanian Veterinary Academy (LVA). Boars were assigned to the groups according to their age (from 7 to 34 months of age). In total, the quality of 416 ejaculates was assessed. There were analyzed the influence of boar breed, age and season on semen quality parameters. Statistical analyses were performed using SPSS statistical package. The difference was recorded to be statistically when p Ò0.05.

We found that the breed of the boar had a significant effect on volume of the ejaculate, concentration of spermatozoa and percentage of pathological spermatozoa ($p<0.001$). The breed of boar had not significant effect on sperm motility. Compared to the boars of Duroc breed, Danish Landrace boars had higher volume of ejaculate, but lower sperm concentration of spermatozoa. Danish Landrace boars had lower incidence of pathological spermatozoa in their semen, compared to the boars of Duroc breed.

Age of animal also had a significant effect on volume of the ejaculate, motility, concentration of spermatozoa and the incidence of pathological spermatozoa ($p<0.05$). We found the volume of the ejaculate increases with the age of the boar. With the advancing age, volume of ejaculate increases either increases the volume of ejaculate but concentration of spermatozoa decreases. The highest percentage of motile spermatozoa was found in boars that during semen collection were 18–24 month of age, and the lowest – in boars that were older than 30 month of age. The highest incidence of spermatozoa with pear shaped heads was found in boars younger than 12 month of age, and the highest incidence of spermatozoa with simple bent tails was found in boars that were over 30 month of age.

Season had a significant effect on sperm motility, concentration and number of pathological spermatozoa ($p<0.001$). Season had not significant effect on volume of the ejaculate. Sperm motility decreased during summer and autumn, and sperm concentration decreased during autumn and winter. The lowest incidence of pathological spermatozoa was observed beginning of summer. The highest incidence of pathological spermatozoa was observed in the end of summer and beginning of autumn.