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TABLE OF CONTENTS

The Seroepidemiological Survey On The Spread Of Bovine Viral Diarrhea Virus (BVDV) In Estonian Cattle Herds — *A. Viltrop, J. Alaots, T. Laht, L. Ronsholt* 3

Bovine Leukosis In Lithuania — *J. Glamba, D. Apanavičiene* 8

Immunogenetic Aspects Of Resistance And Immunity — *Ü. Pavel, A. Viltrop*..... 11

Investigation Of Contagious Diseases In The Tartu Veterinary School And Tartu Veterinary Institute — *E. Ernits* 14

On The Mechanism Of Alimentary Acid-Base Balance Disorders In Cows — *K. Kadarik* 16

About Causes Of Pre- And Early Postnatal Mortality In Calves And Pathological Changes Found In Them — *N. Koslov, E. Lepp, V. Lokk, J. Song*..... 23

Ultrasound Guided Puncture Of Follicles In Heifers — *J. Kurykin, H. Gustafsson, B. Larsson, H. Rodriguez-Martinez* 30

Evaluation Of The Corpus Luteum Activity In Synchronized Recipients On The Day Of Embryo Transfer — *J. Kurykin*..... 34

Helminths Of The Most Important Game In Estonia — *T. Järvis*..... 38

Ultrasonic Measurement Of Animal Eye Dimensions — *M. Paunksniene, A. Paunksnis, V. Babrauskiene, A. Lukosevičius*..... 42

Isolation Of Canine Parvovirus In Cell Culture And Investigation Of Its Properties During Experimental Infection — *A. Stankevičius, A. Šalomskas, L. V. Belianko* .. 45

An Effect Of Ferolit On The Organism Of Animals — *J. Žymantiene, B. Girnius, Z. Beresnevičius, B. Sapijanskaite, K. Kraujelis, A. Ulinskaite*..... 49

Research Of New Veterinary Preparations For Prophylaxis And Treatment Of Mastitis In Cows — *V. Špakauskas*..... 54

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What for and for whom?

This rhetorical question probably needs answering every time a new periodical is initially proposed to a wide readership. So this new scientific veterinary journal in English, issued in Estonia, must be also well grounded.

The collapse of the socialist system brought with it big changes in every field of life including science in the former socialist republics that have now become free countries. As the socialist system was a closed society, scientific contacts were only possible between the socialist countries and so the spread of scientific information was restricted to these countries. Information from other parts of the world was scarce and occasional. The main veterinary journals were published in Russian in Moscow. Access to the world-wide distinguished scientific publications was restricted. Also, the principles of veterinary medicine in the socialist system were different from world understanding. The main objects of Soviet veterinary medicine were farm animals. Less attention was paid to pets and this deficiency has now to be eliminated in all the Baltic states. Also, the role of veterinary medicine in the protection of Man from diseases common to animals and Man was not totally accepted. Veterinary medicine was restricted to the agricultural sciences but its role is much larger. Now the time has come to restore veterinary medicine to its proper place in the common line of sciences in all Baltic countries and consciously to promote the fact that veterinary medicine deals not only with farm animals but also with pets and the protection of man.

Up to now the big problem confronting scientists of the previous Soviet republics has been the lack of possibilities for the international spread of local scientific information. The spreading of such information is of vital importance for every scientist because it forms a scientific image not only of the scientist but also of the general scientific level in a certain region. It also informs about successes in the research field as well as having an informative value.

Taking into account the above mentioned, the importance of an informative channel for veterinary scientists of the Baltic region is evident. However, due to financical difficulties we are not yet ready to publish our own regional journal "Acta Veterinaria Baltica" and therefore the Estonian Veterinary Association has decided to publish a volume of the "Estonian Veterinary Review" containing scientific publications of Baltic veterinary scientists in English. This is the first issue. We are sending it to the various veterinary institutions around the world and hope for its benevolent acceptance. With best wishes to our readers and authors in the future.

Jaagup Alaots
Prof, PhD
Editor in Chief

The Seroepidemiological Survey On The Spread Of Bovine Viral Diarrhea Virus (BVDV) In Estonian Cattle Herds

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Abstract

Data obtained from the country-wide serological screening of cattle herds demonstrates the wide spread of the Bovine Viral Diarrhea Virus in Estonia. Randomly chosen herds from every administrative district were sampled (10 heifers in the age of 6—24 months and 10 cows from each). The serum samples were tested with a liquid phase antibody blocking ELISA (SVIV). Eighty six percent of tested large herds were found to be seropositive. Fifty percent of the herds were suspected of having PI-animals as the seroprevalence in the small herd sample from young stock was higher than sixty percent. The BVDV antigen ELISA (SVIV) was used to detect PI-cattle from three suspect herds. All three herds proved to contain PI-animals, thus the serological spot test seems to be a sensitive and suitable method for the detection of PI-herds.

Key words: BVDV, Bovine Viral Diarrhea, persistent infection, seroepidemiology, surveys, ELISA

Introduction

The presumption for the existence of infectious disease is the incessant epizootic process. The epizootic process in turn may only be realized in the presence of the three compulsory links of the epizootic chain: the source of infection, the adequate spread mechanism of the causative agent and the susceptible organism. As a nosologic unit, the infectious disease is not a fixed and stable but continuous and developing phenomenon. The influence of various environmental determinants and the interaction between micro- and macroorganisms makes them change in the evolutionary process and as a result of that the manifestation of the disease changes as well.

The general trend in the evolution of viral infections is the development of symbiotic intercourse between the virus and the host. Therefore, in spite of the large spread of the causative agent in the population, the clinical disease is scarce and mostly due to various stressors weakening the mechanisms of natural resistance. In many animal populations the interaction between micro- and macroorganisms have reached the status of equilibrated biocoenosis (1).

The spread of five viruses — Infectious Bovine Rhinotracheitis Virus, Respiratory Syncytial Virus, Adenovirus, Rotavirus and BVDV — has been asserted by virus isolation from the organ material of dead animals with various clinical syndromes in Estonian cattle herds (2).

The purpose of the present study was to clarify the spread of BVDV among Estonian cattle herds in a large scale serological survey.

BVDV belongs to the family *Flaviviridae* genus *Pestivirus* (3). The virus is spread world-wide and has been shown to cause various problems in cattle herds ranging from abortions and stillbirths, weak calfs and neonatal calf mortality to diarrhea and respiratory disease in young stock (4). The fatal mucosal disease caused by BVDV is sporadic and limited to persistently infected (PI) cattle (5). It has been postulated that the BVDV also has an immunosuppressive impact on the host as the virus causes the defective function of leukocytes (6). Therefor the BVDV infection may be a cause of acute outbreaks of other viral and bacterial infections (4).

The crucial phenomenon in BVDV epidemiology are PI-animals who are the main sources of the virus spread in the herd. They are thus responsible for the maintenance of the infection in the herd and the main factor in the spread of the infection from herd to herd (7). It is therefore important to eliminate these animals.

It has also been demonstrated that the herds containing PI-animals (PI-herds) can be identified by serological testing of only a few animals in the herd (8,9).

The second purpose of the study was to learn if serological screening like this is suitable for Estonian large dairy herds, where cows and young stock are often kept in several separate units.

Material and methods

Serum samples available for the BVDV screening of the Estonian cattle herds were obtained from the Regional Veterinary Laboratories running the national eradication program for Enzootic Bovine Leukosis.

The Estonian cattle population was distributed

roughly into two distinct groups of herds- small herds (1—50 cows) and large herds (more than 50 cows). In 1993 there were about 40,000 small herds with 80,000 cows and 700 large herds with 145,000 cows.

Due to restricted funding it was decided to investigate only a fraction (20%) of the large herds, randomly chosen from each of the 15 Estonian administrative districts, and a smaller fraction (0.5%) of the small herds from the same districts by a serological BVD spot test. Altogether 176 large herds and 159 small herds were examined (Table 1 & 1A).

Table 1. *The results of the serological spot test for BVDV antibodies in Estonian large cattle herds.*

District	Number of herds tested	Infected herds	
		Number	Percentage
Harju	9	8	88.9
Hiiu	3	3	100
Ida-Viru	10	8	80
Jõgeva	10	9	90
Järva	21	17	80.9
Lääne	9	9	100
Lääne-Viru	12	10	83.3
Põlva	9	7	77.8
Pärnu	10	8	80
Rapla	9	6	66.7
Saare	19	8	88.9
Tartu	16	15	93.8
Valga	23	23	100
Viljandi	15	12	80
Võru	11	8	72.7
Total	**176**	**151**	**85.8**

Table 1A. *The results of the serological spot test for BVDV antibodies in Estonian small cattle herds.*

District	Number of herds tested	Infected herds	
		Number	Percentage
Ida-Viru	71	25	35.2
Jõgeva	4	1	25
Järva	12	6	50
Lääne	11	7	63.6
Lääne-Viru	1	1	100
Põlva	30	11	36.7
Rapla	6	1	16.7
Saare	3	3	100
Võru	21	10	47.6
Total	159	65	40.9

Table 1B. *The results of serological BVDV screening of 3 Estonian AI-stations.*

AI-station	Number of animals tested	Infected bulls	
		Number	Percentage
Arkna	10	5	50
Kehtna	12	11	92
Märja	10	9	90

From 113 of the large herds each spot test consisted of serum samples from 10 older cows and from 10 animals between the age 6—24 months (Table 2), while the sample from the remaining 63 large herds was collected only from cows. The number of small herds investigated was 159 and the sample normally consisted of serum from less than 10 animals within all age groups. A serological investigation of 3 A.I. stations was also performed by a spot test consisting of serum samples from 10—12 bulls from each (Table 1B).

Table 2. *Presence of large herds suspected of housing PI-animals on the bases of serological spot test from young stock.*

District	Number of tested herds	Number of sero-positive herds	Number of suspected PI-herds	Percentage of suspected PI-herds*
Harju	3	2	2	66.7
Hiiu	3	3	0	0
Ida-Viru	10	7	3	30
Jõgeva	8	7	6	37.5
Järva	8	7	2	75
Lääne	9	9	6	22.2
Lääne-Viru	7	6	4	57.1
Põlva	7	7	6	85.7
Pärnu	4	4	3	75
Rapla	9	6	2	22.2
Saare	8	7	5	62.5
Tartu	6	6	3	50.0
Valga	8	8	5	62.5
Viljandi	15	12	11	73.3
Võru	8	7	1	12.5
Total	113	98	56	49.6

* Calculated against number of tested herds

The serological investigations were performed by a liquid phase antibody (Ab) blocking ELISA (SVIV) used in the Danish BVD eradication program. Briefly, a known amount of BVDV antigen (Ag) giving an OD value of 1.0 and the serum samples or dilutions hereof was incubated (24 h) in wells precoated with swine anti-BVDV Ig G. After a washing procedure, BVD detection polyclonal rabbit serum, peroxydase enzyme-conjugated anti-detection Ig G and substrate was added. The decrease (blocking) in the developed colour reaction (OD value) compared with a known BVD Ab negative serum was calculated. Fifty percent or more blocking was registered as the positive reaction.

Herds containing more than 60% seropositive animals among the young stock were suspected of housing BVDV persistently infected (PI) animals (Table 2)

In three large herds classified as suspected PI-herds, the young stock was individually tested for the presence of PI-infection by an Ag ELISA (SVIV). The dams of detected PI-animals were also tested for BVDV PI-infection, and the bodyweight and growth gain of these PI-animals was compared with those of a similar age.

The BVDV Ag ELISA (SVIV) was performed according to the above mentioned principle for the Ab ELISA. Briefly, the leucocyte fraction of a heparin stabilized blood sample was separated (10) and treated with a detergent before being added to 4 wells of a microplate coated with swine anti-BVDV Ig G. After an incubation of 24 h followed by a washing procedure, 2 of the wells were incubated (0.5 h/37 °C) with rabbit

anti BVDV detection serum and the 2 remaining wells with normal rabbit serum. Finally following a new washing procedure, peroxydase enzyme-conjugated swine anti-rabbit IgG and substrate was added. The positive reaction according to the spectrophotometric reading was a three-fold higher OD value (0.1) of the test wells compared with the control wells.

Results

The serological investigation for BVDV antibodies demonstrated that respectively 85% and 41% of the large and small cattle herds as well as the bulls of the A.I. stations in Estonia are or have been infected with BVDV (Table 1, 1A, 1B).

In part of the large herds where the serological spot test was performed on serum samples from 6—24 months old animals, the positive reaction will demonstrate recent spread of the BVDV infection (Table 2). Out of 113 of these herds 98 (87%) had experienced the infection and 56 of them (50%) were to such a level (60% seropositive animals) which could indicate the presence of one or more PI-animal.

The distribution of herds based on seroprevalence in the small herd sample, taken from young stock of the herd is presented on Figures 1 & 2. This advocate for the mentioned threshold value of 60% , above which a herd may be suspected of containing an infectious source such as a PI-animal.

A positive serological spot test from older animals may not represent the actual BVDV situation, anyhow in most cases the seroprevalence among cows in PI-suspected herds is also high (Figure 3).

In order to prove that a BVDV seroprevalence of more than 60% among the young stock may demonstrate the presence of one or more PI-animals in the herd, the young stock was individually tested for BVDV infection by the Ag ELISA. All three herds proved to contain PI-animals (Table 3). However, the PI-animals were often limited to one unit of the farm, although the other non contaminated units also demonstrated a high BVDV seroprevalence.

10 PI-calfs (persistency of the infection confirmed by retesting after 1 month) were discovered. None of them had antibodies to BVDV.

Five dams of PI-calfs had survived and one of them happened to be BVDV antigen positive and seronegative. Four others were antigen negative and seropositive. The bodyweight and yearly milk yield of the PI-dam was normal and close to the mean of the herd.

The body weight of PI-yearlings was on average 44 kg less than their age mates. In the age of 20 months the difference was on average 83 kg.

Discussion

The results of the present study have proved the wide spread of the BVDV amongst Estonian cattle — 65% of all tested herds occurred to be seropositive. The impact of the herd size to the virus spread is evident — 86% of large herds and only 41% of small herds were seropositive. In spite of the fact that the sample size on the district level was not representative to demonstrate the proportion of infected herds in each of them, it is most likely that there is no big differences between different regions. Only in three districts were less than 80 % of the tested large herds affected (see Table 1).

The results from AI-stations showed the possibility of the BVDV spreading due to PI-animals among breeding bulls. As BVDV is excreted with and capable to persist in frozen semen this may cause infection in susceptible cattle at insemination (11). It is therefore important not to use PI-bulls for semen collection. Hence an obligatory screening of all the bulls in AI-stations for BVDV persistent infection is necessary.

The distribution of herds according to seroprevalence in the small sample taken from cattle in the age group 6—24 months, displayed on the histogram in Figure one, brings out two different groups among large herds: group-A — where the seroprevalence in the herd sample ranges from 0—60%; and group-B — where it is higher than 60%. That coincides well with the results received by H. Houe (9). Only a few herds have stayed in the "grey zone" (50—70%). The box and whisker plot presented in Figure two based on the same data show that the middle half of the herds in group-A have seroprevalence in the herd sample in the range of 0—20%, and the same for group-B is 80—100%. Thus the differences are clear and the different epidemiologic situation in the two groups can be expected.

The seroprevalence in the herd sample taken from cows of the group A herds is irregular. In many herds high prevalence has been observed (see Figure 3). At the same time he seroprevalence in the herd sample is low only in a few herds classified as PI-positive. That demonstrates the active spread of the virus in group B-herds in every age group.

In total, 50% of large herds had elevated seropositivity among young stock. Due to the small sample size at district level, the differences between districts coming out from the data presented in

Table 3. *The BVDV antigen detection in three large herds.*

Herd number/ unit number		Number of animals tested	Age (months)	Seroprevalence in the spot sample %	Number (%) of PI-animals
I /	1.	314	6—16	100	8 (2.5)
	2.	340	16—24	100	0
	3.	46	16—24	100	0
	4.	39	24—36	100	0
		15	0—2*	X	0
Total		**754**	**X**	**100**	**8 (1.1)**
II /	1.	119	8—24	40	0
	2.	115	8—16	60	0
	3.	263	8—24	100	1 (0.4)
	4.	155	8—24	60	0
	5.	125	8—24	80	0
	6.	131	8—24	50	0
Total		**908**	**X**	**65**	**1 (0.1)**
III		**53**	**0—24**	**90****	**1 (1.8)**

* *age in weeks*

** *in age group 6—24 months*

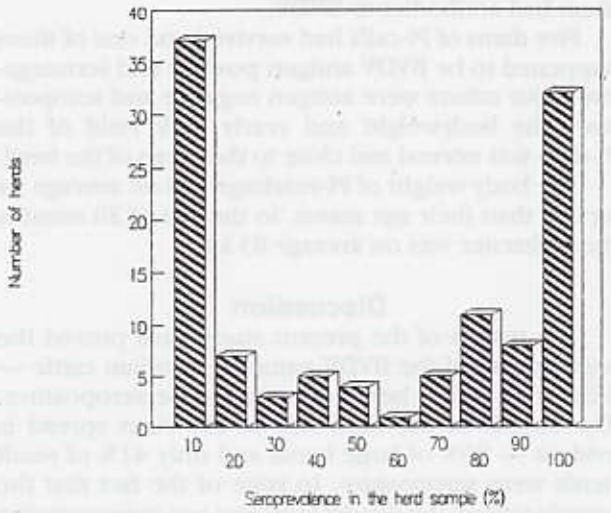

Figure 1. *Distribution of herds by seroprevalence in the herd sample taken from young stock (113 herds included).*

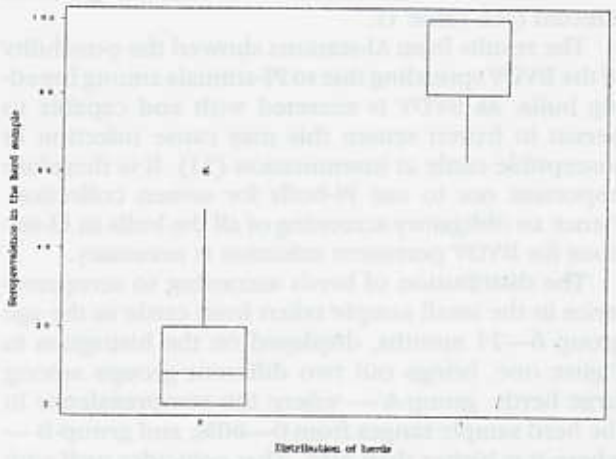

Figure 2. *Distribution of herds in PI-non-suspected (0) and PI-suspected (1) herd groups by seroprevalence in the herd sample taken from young stock.*

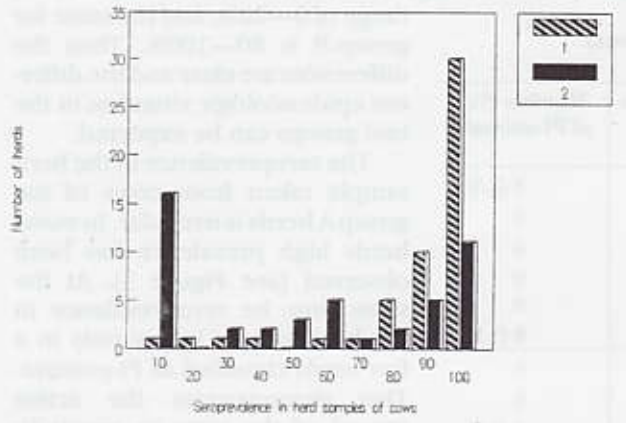

Figure 3. *Distribution of BVDV seroprevalence in a herd sample of cows in 113 herds suspected (1) and not suspected (2) of having an acute BVDV infection caused by a PI-animal.*

Table 2 are not plausible.

In all three large herds classified as PI-positive and tested for virus carriers, the PI-animals were discovered. That confirms the sensitivity of the spot test. However, in herds 1 and 2 in some units where the seroprevalence in the herd sample was higher than 60%, no PI-animals were discovered. That can be explained by "natural" selection — PI-animals could be slaughtered because of retarded growth or have died because of bad resistance to diseases.

The serologic investigations in herd two have demonstrated that in some cases the presence of PI-animals in the herd does not result in high seroprevalence, that in the case when the herd is divided between several units and young stock from one shed has no contact with animals from other units.

Conclusions

BVDV is widespread in Estonian cattle. The large herds are up to twice more infected than small herds. This fact emphasizes once more the essential role of the herd size in spread of pathogens and confirms the importance of horizontal transmission of BVDV. That also confirms that the epizootic process in different conditions proceeds differently and stresses the need to pay more attention to veterinary hygiene in the case of industrialized animal husbandry.

The serological testing of the small herd sample is a sensitive method for screening of PI-positive herds.

To discover the PI-animals in the suspected herd all young stock should be tested for the virus antigen using ELISA (retesting of antigen positive animals after three weeks to confirm the persistence of the infection). Also the dams of PI-animals should be tested for the persistent infection, as sometimes they can grow as well as normal cattle and give offspring.

When the animals of the herd are divided into several separately managed units, each unit of that kind should be handled as a separate herd.

The epidemiology of BVDV in large herds and the impact of the infection on the health status of the herd needs further investigation.

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Bovine Leukosis In Lithuania

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Abstract

Bovine leukosis was first registered in Lithuania in 1916. Isolated cases of the disease were diagnosed in the period from 1936 to 1962. Wider spread of the disease was detected on farms and slaughterhouses after 1965. 7.6 thousand cases of bovine leukosis were registered in 1971—80. In this period patologicoanatomical changes characteristic of bovine leukosis were stated in approximately 85—130 carcasses out of 100 thousand at the slaughterhouses of the Republic. Since 1987 serological tests — agar-gel immuno-diffusion test (AGIDt) has been used alongside hematological tests in leukosis diagnostics. In 1988, after having tested 852.9 thousand cattle on farms, 35.6% carried leucosis virus, 43.2% of them being cows. The high number of infected animals were discovered in Šiauliai and Panevezys districts, while the lowest was on the farms of Klaipeda district.

Key words: Bovine leukosis, epizootology, haematological, serological, pathomorhological diagnostics, slaughterhouses

Bovine leukosis is the most widely spread infectious disease in Lithuania. It brings about enormous economic losses due to early cow rejection, setbacks in selection work and use of young animals for breeding and need for expensive veterinary-preventive measures.

After having established the ethiology of the disease, both serological diagnostics and strict administrative farming regulation were applied, resulting in the stabilization of the epizootic status of the disease in Lithuania and cutting down the sickness rates or even eliminating the disease on some farms.

In the research of bovine leukosis, it is essential to have a clear view of the epizootic situation, that is, to evaluate the spread of the infection.

A number of authors (1, 2, 3, 4) have analysed the ways by which the infection spreads and some other epizootic problems.

Every year numerous blood samples are tested on Lithuanian farms, therefore our knowledge about the epizootic situation changes and it must be constantly analysed and updated. Taking all this into account, we have analysed and described the bovine leukosis epizootic situation in Lithuania from the very beginning to the most recent investigations. For this purpose, a number of literary sources, archives and reports on the diagnostic tests have been studied.

The first cases of bovine leukosis were detected in Lithuania in Klaipeda district in 1916 (5), after which no new cases were registered for 20 years. From 1936 to 1940 several cases of the disease were diagnosed at the clinical hospital of Lithuanian Veterinary Academy and in Panevézys district. Then several breeding animals were imported from Germany, Denmark and some other European countries where this disease occured. Probably some infected animals were brought to Lithuania in this way. Most often they were bought by large farms and estates.

In the first post-war decade, no cases of leukosis were registered, while in the period from 1955 to 1966 single cases of the disease were diagnosed in Šiauliai, Radviliškis, Jonškis and Kaunas districts. As no haematological testing of cattle was practiced in Lithuania at that time, the exact epizootic situation of the disease is not known. In 1961—1962 more frequent cases of leukosis were diagnosed and a greater number of changes characteristic of leukosis were found at slaughterhouses. In 1963 15 cases of leukosis were diagnosed out of the total 546 cattle tested haematologically on some farms. To get a clear view of the epizootic situation in Lithuania, all the cattle on state, collective and breeding farms were tested in the period from 1964 to 1968, and from 1970 also all those privately owned animals were investigated. In the period from 1966 to 1970 8.1 thousand cases of leukosis were diagnosed on 138 farms. It must be noted that the morbidity found were highest on state breeding stations and farms.

In 1971 the morbidity started to decrease and in the seventies the number diagnosed was 7.6 thousand.

In the period from 1966 to 1970, leukotic tumours were found in 3.3 thousand animals at slaughterhouses. From 1971 up to 1975 this number dropped to 2.7 thousand per year, while in the period from 1976 to 1980 it grew again, reaching up to 4.1 thousand cases per year. In 1981 to 1985 the morbidity reached 4.5 thousand cases per year. The incidence of leukotic tumours was 85—130 cases per 100 thousand slaughtered cattle. In the period from 1981 to 1985 the results of the hematological tests showed a decrease in morbidity, averaging at about 110 to 650. These were the official results presented in statistics, but actually the numbers both of the farms and the cattle affected were considerably higher.

From 1986 to 1989 leukosis cases increased drastically. In 1987 the number diagnosed on farms haematologically was 2777 out of 590 thousand, that is 0.4%,

in 1988 this number reached 12.8 thousand (1.5%). The peak was reached in 1989 at the number of 25.6 thousand (3.2%). Since then a gradual decrease has been observed; 1990 — 25.2 thousand, 1991 — 19.8 thousand, 1992 — 132 thousand, 1993 — 78 thousand and 1994 — 5.8 thousand.

Leukosis was less frequent among privately owned cattle than that on state and communal farms. In 1988 out of 196.3 thousand haematologically tested cattle 1435 (0.7%) cases were diagnosed, in 1989 — 3515 (1.6%). In 1990 — 2402 (2.3%) cases and in 1992 1417 (2.2%).

Figure 1 presents the results of haematological testing of cattle for leukosis in Lithuania in the period from 1986 to 1994.

Since 1987 serological blood testing has been started alongside with hematological tests. Cattle, infected with leukosis virus, were diagnosed by means of agar-gel immuno-diffusion reaction (AGID). In 1988 852.9 thousand cattle were tested on farms and 35.6% of them were seropositive (43.2% of this number being cows). In 1989 — 1.317 thousand were tested, the percentage being 22.7% and 30.6% respectively, while by January 1, 1991, the number had reached 60.7% of the total number of 505.7 thousand of community cows tested. The following years brought a gradual drop in seroprevalence, resulting in 39.8% in 1992 and 27.9% in 1994.

The disease and infection rates differ in various places of Lithuania. The most affected areas are Šiauliai and Panevezys districts, while the rates on the farms of Klaipeda district are the lowest. The infection rates, on community farms in the period from 1988 to 1989, are presented in Figure 2.

The infection rates of serologically positive cows of privately owned cattle are slightly different. 128.7 thousand cattle were tested in 1988 and 25.1 thousand (19.5%) were found to be infected with leukosis virus. In 1989 the number was 69.8 thousand (18.4%) of the 379.6 thousand tested and in 1990, after having tested 368 thousand cattle, the number was found to be 46.5 thousand (12.6%). This number continued to decrease, reaching 10.1% in 1991; 8.6% in 1992, while in 1994 it reached the lowest rate of only 5,1%.

The highest number of cattle infected with the leukosis virus has been found in Panevézys, Šiauliai and Vilnius districts, while the lowest one has been on the Klaipeda district farms.

Historically, it is known that bovine leukosis was first diagnosed in Germany at the beginning of the century, while in Denmark and in Holland it was found in the first years of the post-war period (5.6). Breeding animals were imported from the abovementioned countries to Lithuania even before 1940, and was bought by big farms and estates as a rule. In the summer-autumn of 1945 thousands of cows were being driven through Lithuania to other Soviet Republics from the Kaliningrad district, where frequent cases of cattle leukosis occurred in those days. A number of these cows were bartered with the local people or sold to them and thus stayed on our farms. In the post-war period quite a number of bulls and heifers were brought to Lithuanian state and breeding farms from the above mentioned countries. Therefore it is fair to maintain that bovine leukosis could have been

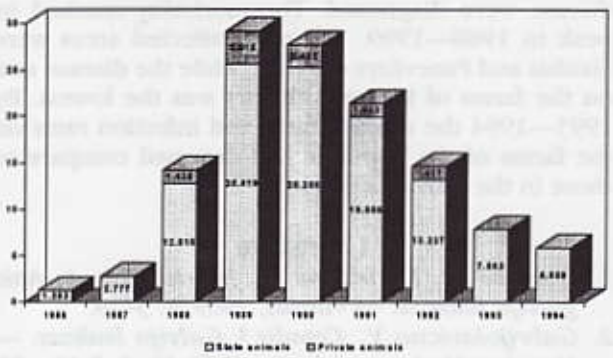

Figure 1. *Number of BLV hematological positive cattle during the period 1986—1994.*

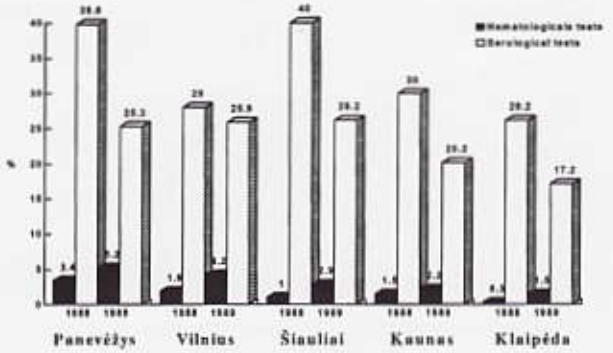

Figure 2. *Bovine leucosis in various areas of Lithuania in the years 1988—1989 (community cattle).*

brought to Lithuania through the channels mentioned above.

Before the first tests were made in 1963 the disease had been quite wide spread in some parts of Lithuania. By 1975, 3.36 million heads of cattle had been tested and more than 12 thousand cases of leukosis diagnosed, with all the sick cattle having been taken to slaughterhouses. From 1976 to 1980 these tests were carried out on a regular basis, all the sick cattle being isolated and slaughtered. In the period after 1980 this work was neglected. Moreover, in an attempt to conceal the real state of affairs the number of sick cattle used to be cut down, so that the statistical data of those times presented markedly lower morbidity and therefore cannot be relied on. As a result was a much larger of the disease in farms. It is well-known that with use of haematological tests alone is not possible to completely eliminate the disease, as a great number of infected cattle cannot be discovered and they fall ill later on. Therefore, after having tested our livestock in 1989—1990, 56.7 thousand cases of leukosis were found in total.

It must be noted that in recent years the number of cases both on individual and community farms has markedly decreased. On some community farms no cases of the disease have been found recently. The new program of measures against the disease aims at minimizing morbidity in the near future.

Conclusions:

The first case of bovine leukosis was registered in Lithuania in 1916. Until 1962 only single cases of the

disease were diagnosed. The morbidity reached its peak in 1988—1990. The most affected areas were Šiauliai and Panevėzys districts, while the disease rate on the farms of Klaipeda district was the lowest. By 1993—1994 the disease cases and infection rates on the farms of the Republic had dropped compare to those in the previous years.

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Immunogenetic Aspects Of Resistance And Immunity

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Abstract

The paper deals with the interpretation of the terms resistance and immunity, immune response or immunoreactivity and the way of measuring the degree of endurance of an animal. It presents a simple scheme of the relationship between immune response and immunity or resistance.

Key words: endurance index, resistance, immune response, immunoreactivity, herd, population immunology

Having in mind the different terms used in immunology to designate similar or even identical phenomena, we proposed (Pavel, 1971) a scheme in which we tried to present immunological phenomena in a logical sequence, where an argument (independent variable) is followed by its function (dependent variable). In this case the reactivity (reaction) of an organism as an argument will serve, and its function will be the result — the resistance or immunity (or susceptibility).

The scheme (a little amended) is as follows:

A. Immunological reactivity, immunoresponsiveness or immune response (recognition of the antigen and the reaction produced).

1. Non-specific reactivity (determines the non-specific response of organism). It depends on the individuality (structure and function) of (a) nonspecific factors of resistance (barriers, humoral factors etc.); (b) systemic factors (endocrine and nervous systems); (c) the absence or presence of a certain metabolite and/or adhesion receptors on cell membranes.

2. Specific reactivity (determines the immune response). It depends on the individuality of lymphoid apparatus.

B. Resistance or immunity, the function of immune response; it does not always depend on the strength (sign; + or –) of the argument; it can be subdivided: (a) non-specific resistance (depends on the non-specific reactivity); (b) immunity or specific resistance (depends on both forms of reactivity), and (c) resistance based on hyporeactivity of the individual. The non-specific (1) and specific (2) immunological processes may appear in the following combinations: (a) (1) and (2) have the same direction (individuals with high, resp. low non-specific and specific reactivity); (b) (1) and (2) have different directions (individuals with high non-specific and low specific reactivity).

Concerning the relationship between the reactivity and immunity or resistance, we have found (Pavel, 1977, p. 136—137) that experimentally infected (with *Salmonella gallinarum*) chicks which and survived had very different amounts of specific antibodies in their blood serum — the titre of 1:8—1:16384.

When studying the ontogeny of immune response and immunity it is necessary to bear in mind Haeckel's law of biogenesis, which presumes that at early stages of individual development the cellular factors of immunity ought to be expressed more extensively than are humoral ones.

When evaluating the resistance of animals on the bases of 2—5 main immunological traits, it is appropriate to use the term "endurance index". We propose to evaluate the endurance by three — five simple traits like longevity (Lon), skin hypersensitivity reaction to phytohaemagglutinin (PHA), bactericidal activity (BAC) of blood serum to *Escherichia coli* O127 and *Micrococcus luteus*; the total protein content of the blood serum (Prn): the morbidity per one lifeyear (Mor, inflammatory and metabolic diseases), and fertility (Fer, the number of inseminations needed per conception.

Some modern terms used in immunology must be born in mind: multiresistance and immunocompetence. The first designates the resistance of a genotype to some or several antigens and allergens, and the second means a state of "polyimmunity" (responses to various pathogens or antigens).

In domestic birds, one is also able to evaluate polyimmunity as multiresistance. But, for practical reasons, it is possible to determine only the degree of immunocompetence (i.e. polyimmunity) in large farm animals.

In small animals (such as mice) and chicks it is possible to evaluate the multiresistance of a line. Doing this it is reasonable to proceed from the idea of Van Loveren (1995), who used five pathogens (*Listeria monocytogenes, Streptococcus pneumoniae*, cytomegalovirus, influenza virus, *Trichinella spiralis* and *Plasmodium berghei*) in mice for the infection in researching the resistance models for immunotoxicants.

In large animals it may be convenient to use the side relatives, not only the offspring.

The immune response to the pathogen or antigen is due to the non-specific and specific defence reactions. But the low immunological reaction (immune response) does not always cause the low degree of

resistance, i.e. the animal's susceptibility to pathogen. This controversy may be conditioned by the deficiency of regulator substances, i.e. the defectiveness of cytokine production (its hypo — or hyperproductivity) (see Myers and Murtaugh, 1995).

Almlid (1981) suggested that one has to test animals by challenging them with various antigens and then the genotype's ability to give an immune response may be investigated. However he continued, "the main problem is to find antigens which are well suited to give a "general picture" of the power of the specific immune response".

The situation is aggravated by the circumstance that the immune response is a polygenic trait and the immunological traits are "mobiles" (R.V. Petrov, 1983); so there are also negative correlations between the various immunological traits (Biozzi et al., 1979; Praks, 1983). Therefore one has to select carefully the main immunological traits which reflect the immunological potentialities of the animals as completely as possible, and the selected 2—5 major traits should be measured with express-methods.

Are there any "universal genes" that act in many or in all immune responses to various antigens? Are there antigens which induce the "generalized" immunocompetence? According to Benacerraf and Katz (1975) the different MHC (major histocompatibility complex) haplotypes react differently to various antigens. Bacon and Dietert (1991) emphasize that the genes of "generalized action" are (1) genes that impact an acquired immunity but are not antigen-specific (i.e. cytokine genes), and (2) genes that influence innate immune responses (inflammation, serum bactericidal activity, bactericidal substances of epithelial and other cells, etc.).

So, according to the opinion of Bacon and Dietert the "generalized" immunocompetence is partly based on acquired and innate immunity. This opinion is affirmed also by findings of Pitcovski and coworkers (1987), that vaccination in early perinatal development with Escherichia coli impacts also the immune traits to other antigens.

It is reasonable to call attention not only to the age of the recipient animal but also to the used strain of opportunistic microorganisms as *E. coli*, which induces the "generalized" immunocompetence perhaps only in young developing organisms (see Goren, 1978).

Already in 1944 V.I. Ioffe used in the aim of the assessment of the degree of human's "general immunological reactivity" the anti-species antiserum. In cattle it was first performed by G.M. Ivanova (1953).

Is it possible to evaluate also the degree of herd immunity (or immunocompetence)? The answer is yes. But the situation is aggraviated by the fact that in the different ontogenetic stages are activated different resistance genes (see Goren, 1978). This opinion was proposed by Cassell and coworkers (1993). They observed that the animal's survival in early and advanced ages is different.

The survival of population is regulated by the immunocompetence of individuals (Lochmiller et al, 1994), which is influenced by the enviroment (Lochmiller, Dabbert, 1993). They measured the immunocompetence of rodents (*Sigmodon hispidus*) by the antibody synthesis capacity of splenocytes and T lymphocyte response to the mitogen.

In the natural populations of rodents the life history is determined by adaptations, such as survival and fecundity. These complex traits are controlled by the gene clusters (see Stearns, 1994). According to Dingle (1990) the fitness of organisms is founded on these adaptations (life histories). Life history characters covary and function together.

In 1980 we proposed to measure the non-specific resistance index in chicken by evaluating the bactericidal activity of blood serum, phagocytic activity of pseudoeosinophiles, and later the haemoglobin content of blood (Hb) and also the serum titer of interferon (see Pavel et al., 1980; Pavel, Peterson, 1989).

Almlid (1981) recommends the determination of an overall index of non-specific and specific antimicrobial index in cows and bulls. He emphasizes that there has to be determined phenotypic and genotypic correlations among resistance traits, and the relationship between the bull's antimicrobial power and the frequencies of infectious disease of their female relatives in dairy herds.

Below we briefly discuss the problem how to enhance the herd immunity to facultative (potential) pathogens. It may be carried out by administrating the cytokines and immunostimulators, and also by accomplishing the veterinary selection. It would be emphasized that this term was brought into use by Ukrainian scientist Eysner in 1981. He comprehended under veterinary selection the elimination of sublethal traits and immunologically weak individuals. Veterinary selection is carried out in Germany (Distl, 1990), France (Ollivier and Renijo, 1991) and in other countries. Today it is put into practice in Scandinavian countries, Germany and other countries. As an example, we refer to the work of Israeli scientists (Heller et al., 1992) who select the broilers for early immunocompetence.

We would like once more to deal with the importance of the strain of pathogen in selection. We are of the mind that it is reasonable to select livestock to one concrete obligatory pathogen or to improve the reactivity of animals to the facultative pathogens (opportunists). The assertion that the effect of vaccination is not sufficient is not convincing because the new vaccines contain cytokines and immunostimulators (Myers and Murtaugh, 1995 and others). Breeding for disease resistance has been justified having in mind a concrete pathogen (Hutt, 1958, Ollivier and Renijo, 1991 and others), but it would be alluring to select for non-specific immunity.

Also the vaccinoprofylaxis may not be thrown away or even underestimated. So new technologies offer new possibilities, by adding polyelectrolytes (Petrov, 1985), cytokines (Myers and Murtaugh, 1995) or even the so called "reactive immunization" (Wirsching et al, 1995).

We conclude that it is realistic to select for non-specific disease resistance (multiresistance) and for the specific immune response to a concrete pathogen or only for some pathogens.

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Investigation Of Contagious Diseases In The Tartu Veterinary School And Tartu Veterinary Institute

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Abstract

The paper deals with the contribution of lecturers and postgraduate students of the Tartu Veterinary School (1848—1873) and Tartu Veterinary Institute (1873—1918) to the development of microbiology, immunology and epizootiology.

Key words: history of veterinary medicine, Tartu Veterinary School, Tartu Veterinary Institute, contagious diseases.

Tartu Veterinary School (TVS), established in 1848, and Tartu Veterinary Institute (TVI; 1873—1918) were the predecessors of the Faculty of Veterinary Medicine of the Estonian Agricultural University (Tehver 1931; see also Ernits 1995).

Aetiology of infectious diseases. In 1856 Prof. Friedrich Brauell (1807—1882) found microscopic bacilli ("vibrions") in the blood of men and animals who had died of anthrax. He was the first to establish that they occur in the blood only in the case of anthrax (Brauell 1857; see also Ernits 1977a); therefore they are useful for anthrax diagnostics. Arloing discovered the anthrax bacteria.

F. Brauell (1858) established that in the case of anthrax the bacilli appear in blood 1—3 (maximum 18) hours before the death of the infected animal. Thus he attributed a prognostic significance to their existence.

Prof. E. Zemmers (1843—1906), a disciple of F. Brauell's, founded a small laboratory of microbiology at TVS in 1868. He and his students studied aetiology and other problems of contagious diseases there. E. Zemmers declared that each infectious disease has its causing microbe. He proved all statements from the opponents of microbiological aetiology of infectious diseases to be wrong.

E. Zemmers formulated the following thesis: "All the pathogenic microbes have been saprophytes and they can exist in this form even nowadays; in living organisms they pass only some stages of development." The thesis was based on the following: a) an assumption that contagia come from miasmata; b) data of E. Zemmers etc. concerning polymorphism of the microorganisms; c) a declaration of the pleomorphists on genesis of one kind of microbe from another, and d) a statement of the evolutionists saying that ontogenesis repeats phylogenesis.

In 1869 E. Zemmers was the first one in the world to try to induce anthrax through artificial, cultivated microbes. That year he refuted an assertion of all the researchers about the identity of glanders and syphilis (Ernits 1993b). In 1878 E. Zemmers discovered the microbe which probably causes avian pasteurellosis (Zemmers 1879; Ernits 1976b).

Voldemar Gutmann (1876) dealt with etiological agents of wound infections. Pavel Altukhov (1898) investigated the effect of physical factors on microbes which cause glanders (supervised by K. Happich).

Arthur Laosson (supervised by K. Raupach), Alfred Krajewski, and Ignacy Szantyr (both supervised by E. Zemmers) investigated the contagiousness of dog distemper. Karl Liedemann (supervised by V. Gutmann) dealt with the localization of tuberculosis bacilli in sputum, milk and excrements. Kronid Yemelyanov (1903) took into use Hesse's culture medium for diagnostics of tuberculosis (supervised by K. Happich). In the 1880s E. Zemmers also supervised investigations of Aleksei Arkhangelski, Karlis Ozols, Adolf See and Vladimir Tatarski in the field of the aetiology of infectious diseases (mainly anthrax).

In 1899 Prof. Karl Happich (1863—1923) discovered the mycosis of crayfish. The scientists of Tartu were the first to describe some new manifestations of infectious diseases in the Russian Empire (equine epizootic lymphangitis (Ernst Schröder in Valdai, 1897) and probably colibacteriosis (Voldemar Gutmann in Tartu, 1883)) and in Estonia (canine distemper, malignant catarrhal fever of cattle, infectious diseases of guinea pigs (E. Zemmers, respectively 1875, 1885 & 1886) and pasteurellosis in sheep (Georg Audum, 1888)).

Predisposition of the animals. The ascertainment of animals susceptible to veterinary infectious diseases (inoculation of experimental animals) took its rise in Estonia from the studies made by F. Brauell on anthrax. He proved experimentally that anthrax spreads from humans to animals and from one animal species to another. He found out the protective function of the placenta. The studies of predisposition were continued by E. Zemmers and his disciples (Gothars Šiminš, Rudolf Molkentin, and others) mainly in the field of glanders and tuberculosis.

Pathogenesis and pathological changes of the infectious diseases. Pathogenesis of cattle plague was studied thoroughly by F. Brauell (1862), Jozef Ravich (1864), and E. Zemmers (1882). F. Brauell refutes the

exsudation theory, popular at that time.

Diagnostics of the infectious diseases. Very important achievements were made in TVI in the field of veterinary allergology, namely 1) tuberculin was used for the first time in diagnostics of bovine tuberculosis by Ass. Prof. Voldemar Gutmann (1851—1933), and 2) mallein was invented by the army veterinary surgeon Otto Kalninš (1856—1891; see Gutmann 1890; Kalninš 1891; see also Ernits 1976a, 1981c). In 1910 Fyodor Stolypin dealt with precipitation reaction for diagnostics of glanders (supervised by K. Happich) (Èrnits 1981a, 1981b).

Control of the infectious diseases. In the middle of the 19th century it became clear in Russia that the measures taken by veterinary police to stop infectious diseases do not result in steppes, therefore, the immunization of the cattle was recommended there (Jessen 1852; see also Ernits 1994). Prof. Peter Jessen (1801—1875) fought for vaccination actively up to his death, but the time was not ready for revolutionary changes in immunology. P. Jessen was the founder of the so called plague stations where the successful research on this disease was carried out (Ernits 1977b, 1991).

Prof. Friedrich Unterberger (1810—1884) already doubted the suitability of vaccination against cattle plague in 1854. He considered the compulsory slaughter of sick animals and the prevention of any contacts between the healthy and the sick animals to be the best preventive measures (Ernits 1982).

During the last quarter of the 19th century, postgraduate students (Gustav Grünwald, Lev Ivanov, Hugo Warrikoff a.o.) studied resistance of microbes causing infectious diseases to various disinfectants (supervised mainly by E. Zemmers).

The second half of 19th century was a revolutionary period for the development of microbiology, epizootiology and immunology; therefore the majority of the investigations, made in TVS and TVI, dealt with these branches and they were mostly successful.

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On The Mechanism Of Alimentary Acid-Base Balance Disorders In Cows

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Abstract

When non-lactating and non-pregnant cows were fed with rations energetically corresponding to maintenance requirements, feed dry matter protein and crude fibre content variations were accompanied by changes in acid-base status. When rations with a shortage of dry matter protein and crude fibre were fed, on the basis of urine indicators' changes in acid-base status, acidosis shift occurred. In urine pH, BAR and NABE were low and NH_4-content high. In the case of rations with excess protein and crude fibre the above-mentioned changes were opposite in urine and in acid-base status alkalosis shift occurred. Rations with excessive protein and crude fibre increased SBC-content, which refers to alkalosis shift. With a low feeding level alimentary factors cause significant acid-base indicators' changes in urine.

Introduction

In investigating the metabolic condition in Estonian cattle herds, metabolic alkalosis in the cows' extracellular environment was established (L. Valge, 1968, K. Kadarik 1993).

G. Lachmann and M. Schäfer (1985) have supposed the acid-base status in ruminants to be influenced by various nutritional components as in non-ruminant.

Carbohydrates taken in with feed are decomposed with the help of microbes into organic acids in rumen, and most of the protein into ammonia. In the case of the unfavorable composition of feed rations there might arise the danger of acid or alkalic load. Rumen acidosis and consequent metabolic acidosis are caused by rations with excess energy and too little coarse fodder content. Alkalosis may be caused by rations with insufficient energy and crude fibre but which are rich in protein, also by rations with low energy concentration or alkaline feed components or rations with alkaline feed supplements. The last-mentioned authors claim that the essential parameters for evaluating bovine extracellular space acid-base balance are those of rumen fluid and urine acid-base status. The cows of the second lactation 4—6 week p.p. have an average bases-acids ratio (BAR) of 3.3, net acid-base excretion (NABE) on the urine 153 mmol/l, NH4 content 5 mmol/l.

In the case of obvious alkaline feeding anamnesis (defecit of energy and crude fibre), NABE=230 mmol/l, BSR=6.0 and NH4=4.6 mmol/l. In the case of acidosic feeding anamnesis these indicators are: NABE=44 mmol/l, BAR=1.5 and NH4=11 mmol/l.

W. Guder, D. Häussinger, W. Gerok (1987) and D. Häussinger (1990) claim that for synthesis in the liver of urea, ammonia and hydrocarbonate are used in the same amounts as they are generated in decomposing protein.

When ammonia detoxication takes place more with the help of glutamine synthesis, part of HCO_3^- is unused due to the decrease in urea synthesis and is accumulated in the organism causing the alkaline shift in the acid-base status.

The effect of feed ration composition on the ruminal fermentation processes and ruminal fluid pH changes is described by numerous authors.

The data from different papers indicate that metabolic alkalosis proceeds from feeding a ration rich in roughage, or ration of straw treated with NaOH and rich in concentrates and $NaHCO_3$. Alkaline shift in rumen takes place in the case of inactivating ruminal microorganisms, urea poisoning or putrefaction processes. Excessive concentrated feed intake causes ruminal acidosis that can further develop into metabolic acidosis. With feeding rations rich in easily digestible carbohydrates (sugar and starch) the pH of ruminal fluid drops rapidly. Amylolytic microflora becomes predominant in rumen and bacteria decomposing cellulose recede into the background. In addition the rise in the level of volatile fatty acids (VFA) also causes the accumulation of lactic acid and bicarbonate buffer reduction in blood from 24 mmol/l down to 6—8 mmol/l and a rise in metabolic acidosis. Grinding or pelleting the roughage favour the development of rumen acidosis (G. Dirksen 1977, K.-G. Thiemann, S. Rieger and B. Schubert 1992, P. Nørgaard 1993, R. Erdman 1993, M. Murphy 1993, P.D. Møller 1993).

E.Erdman (1993) claims that in ruminants´ regulation of acid-base status by the excretory function of the kidneys is somewhat different from that of non-ruminants. Urine pH (7.0—8.5) in ruminants is higher than in non-ruminants (pH 5—7). In the case of high urine pH, acid-base status is regulated primarily by increasing bicarbonate excretion rate. At low urine pH, there is a decrease in bicarbonate excretion and an increase in the NH_4^+ excretion rate.

Experimental animals, their feeding and methods of study

4 non-pregnant non-lactating cows with rumen fistula were used as experimental animals. Experimental cows were fed individually twice a day. During the testing period the cows' energy need was covered on

the level of maintenance requirement. During the experiment, crude fibre and protein content in dry matter was changed. Data characterizing the rations used are presented in table 1.

Each ration was fed for 7 days and during the last day rumen functioning, blood acid-base status (ABS) and urine parameters were measured. Samples were taken at 4-hour intervals from 7 a.m to 3 p.m three times a day. Blood samples were taken from jugular vein and urine with permanent bladder catheter. Blood ABS indicators were determined with BG-3 device, I.L.,USA. In blood pH and pCO_2 content were measured directly considering blood Hb content and body temperature. Ruminal fluid, blood plasma and urine ammonia content were determined by the method of diffusion. The ruminal fluid pH was measured with a pH-meter, the VFA content in ruminal fluid with a gas chromatograph. Urine acid-base elimination was determined according to F.Kutas (1965) and G.Lachmann and M.Schäfer (1985) titrimetrically by pH-meter. The feed protein content was measured by Kjeldahl and the energy content on the basis of Weende's analysis (Ü.Oll, V.Karis and V.Sikk 1974).

Results

Daily average values of the **ruminal fluid ammonia content** were 3.90...8.30 mmol/l (tab. 1). The lowest NH_4, content 3.90 mmol/l, was observed with ration no.1: with scanty protein and crude fibre. With ration no.3, which was short of protein but rich in crude fibre, the NH_4 content was not essentially higher in ruminal fluid (5.37 mmol/l). Ruminal NH_4 minimum and maximum values were 1.6 and 17.6 mmol/l. A significant NH_4 increase was noted with the protein content increase in dry matter to 18...19% (rations no. 2 & 4, tab. 2, fig. 1,A). Ration no. 2 had a shortage of crude fibre, no.4 was rich in it. Thus rumen fluid NH_4-content was dependent on dry matter protein concentration. The correlation coefficient of these indicators is r=0.447, p=0.001 (fig. 2). There was an analogical relation between protein and energy ratio and rumen NH_4-content (r=0.444, p=0.002).

The daily dynamics of the ruminal fluid NH_4-content by feeding different rations have been presented in figure 3. During the digestion process ruminal NH_4-content changed. With rations dry matter rich in protein (ration no. 2 & 4) the average NH_4 in cows' ruminal fluid at 7 a.m. was 10.4 mmol/l. Ammonia content dropped to 5.55 mmol/l by 3

Table 1. *Experimental cows' feed ration characteristics.*

Ration No.	Feed			Ration contains			
	hay kg	barley meal kg	soya oil meal kg	metabolic energy MJ	dry matter kg	in dry matter: crude fibre %	in dry matter: protein %
1	2.5	3.0	—	49.9	4.715	18.6	10.1
2	2.5	1.7	1.3	52.0	4.713	19.9	19.3
3	5.5	1.3	—	52.2	5.749	27.6	9.4
4	5.2	—	1.5	54.8	5.669	28.0	18.3

Table 2. *Daily average values of ruminal fluid, blood and urine ammonia content by different concentrations of protein and crude fibre in dry matter of the ration (mmol/l).*

No.	Ration: In dry matter PR%	Ration: In dry matter CF%	In ruminal fluid: Daily avg.	In ruminal fluid: Confidence limits (p=0.05)	In blood: Daily avg.	In blood: Confidence limits (p=0.05)	In urine: Daily avg.	In urine: Confidence limits (p=0.05)
1	10.1	18.0	3.90	2.43 5.37	1.53	0.83 2.24	12.21	6.13 18.28
2	9.4	27.6	5.37	3.90 6.84	1.87	1.16 2.57	6.40	0.33 12.47
3	19.3	19.9	8.20	6.79 9.62	1.93	1.14 2.72	5.27	3.68 6.85
4	18.3	28.0	7.24	5.83 8.65	1.63	0.84 2.42	3.77	2.18 5.35

Note: PR — protein, CF — crude fibre

Table 3. *Daily average values of ruminal fluid, blood and urine pH by different concentrations of protein and crude fibre in dry matter (mmol/l.)*

No.	Ration: In dry matter PR%	Ration: In dry matter CF%	In ruminal fluid: Daily avg.	In ruminal fluid: Confidence limits (p=0.05)	In blood: Daily avg.	In blood: Confidence limits (p=0.05)	In urine: Daily avg.	In urine: Confidence limits (p=0.05)
1	10.1	18.0	6.80	6.65 6.96	7.362	7.324 7.401	7.29	7.03 7.54
2	9.4	27.6	7.07	6.92 7.22	7.369	7.331 7.407	8.12	7.86 8.37
3	19.3	19.9	6.86	6.64 7.08	7.372	7.353 7.390	8.08	7.83 8.33
4	18.3	28.0	7.00	6.77 7.21	7.381	7.362 7.399	7.99	7.73 8.24

Note: see table 2

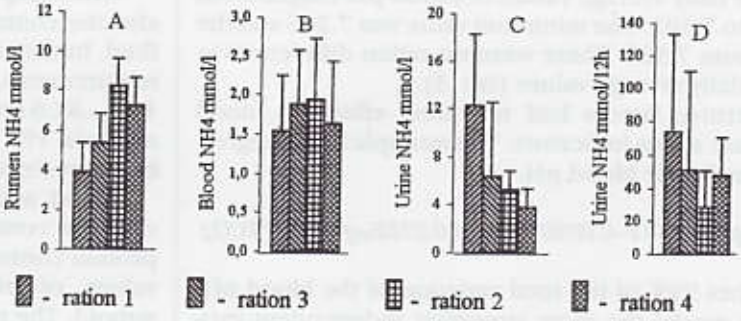

Figure 1. Ruminal fluid (A), blood (B), urine(C) NH_4-content daily average values and NH_4 excretion with urine in 12 hours (D) by different rations

Table 4. *Daily average values of urine bases-acid relation and acids-bases net excretion in ration dry matter with different concentrations of protein and crude fibre.*

Ration			In urine		With urine			
No.	In dry matter		Daily avg.	Confidence limits (p=0.05)	Bases-acids net excretion daily average mmol/l	Confidence limits (p=0.05)	12h acids-bases net excretion daily average mmol	Confidence limits (p=0.05)
	PR%	CF%						
1	10.1	18.0	1.27	0.98 1.57	22.8	3.6 41.9	85.8	−87.3 258.8
2	9.4	27.6	2.01	1.72 2.30	83.7	64.5 102.8	559.7	386.7 732.7
3	19.3	19.9	2.25	1.72 2.78	97.1	57.2 136.9	592.4	419.4 765.4
4	18.3	28.0	2.08	1.54 2.61	71.7	31.8 111.6	581.9	408.9 754.9

Note: see table 2

p.m. When rations with a shortage of protein (no. 1 & 3) were fed, the average NH_4 at 7 a.m. was 4.81 mmol/l. This was significantly lower than with rations No. 2 & 4. With a protein deficit, further changes in NH_4-content were insignificant. When rations with excessive protein and a shortage of it were fed, the average NH4-content of the ruminal fluid at 3 p.m. was not different.

Daily average values of the **rumen fluid pH** with different rations ranged from 6.80 to 7.07 (tab. 3). The minimum and maximum values of the rumen pH were correspondingly 5.82 and 7.50. A difference of rumen fluid in daily average pH values was observed in rations no.1 & 3. Ration no.1 dry matter was short of protein and crude fibre and the daily average value of the ruminal fluid pH 6.80 was significantly lower than in ration no. 3. There was also little protein but excessive crude fibre in the dry matter. Dry matter protein content in rations no. 2 and 4 (18—19 %) was higher than the optimum. With these rations, the increase in crude fibre content did not cause a significant increase in the daily average pH value of the rumen fluid (tab. 3).

When consuming rations with a protein deficit, the daily dynamics of the ruminal fluid pH were similar (fig. 4). In these daily dynamics after feeding at 11 a.m. the drop of the pH in ruminal fluid occured which was not statistically significant. With rations rich in protein, the pH average in the morning was 6.8. Together with the digestion process the pH increased continuously and by 3 p.m. its average reached 7.1. Ruminal fluid pH and NH_4-content daily dynamics were contrary (fig. 3 & 4).

The daily average values of **blood pH** ranged from 7.362 to 7.381. The minimum value was 7.246 and the maximum 7.525. There were no ration differences in those daily average values (tab. 3).

Nutritive factors had no direct effect on blood acid-base status indicators. The multiple linear regression model for blood pH:

$$Blood\ pH = 8.311 - 0.0030 * pCO_2 + 0.07 BE_{ecf} - 0.031 * TCO_2$$

describes 99% of the total variation of the blood pH. In this model the most important independent variable is blood pCO_2 (partial pressure of carbon dioxide) describing 56% of the total variation of blood pH. An increase in pCO_2 causes a pH shift in the acidosis direction.

We observed changes in the pH of blood and ruminal fluid caused by after-feeding digestion and metabolic processes. At 7 a.m. the average pH of the blood was 7.341 and at 3 p.m. it was 7.394. The blood pH alkaline shift before evening feeding was statistically significant (fig. 5). There were no significant differences in the pattern of blood pH daily dynamics registered with different feeding. Also there were no significant differences caused by rations in the pattern of **carbon dioxide partial pressure (pCO_2)** daily dynamics. On average the pCO_2 of all rations was the highest in the morning — 50.4 mmHg and the pCO_2 physiological level was higher in cattle. During the day there was a continuous decrease in pCO_2. At the end of the experiment at 3 p.m. the pCO_2 was significantly lower than the morning level and the average of all rations was 44.4 mmHg (fig. 6). The negative correlation between blood pH and pCO_2 was observed (see figure 7). The pCO_2 values ranged from 38 to 61 mmHg.

No differences caused by feed rations were observed between the **standard bicarbonate (SBC)** daily averages. It was seen from SBC daily dynamics that in the case of rations rich in protein, SBC values varied within 26.5...27.0 mmol/l (fig. 8). Rations with dry matter short of protein were exceptional. Compared to the rest, in the case of ration no. 1 at 7 a.m. there was significantly less SBC — 24.5 mmol/l. In the case of ration no. 3 there was significantly less SBC in the blood at 3 p.m.. SBC values ranged from 21.4 to 30.1 mmol/l and were in negative correlation with blood plasma pCO_2 (fig. 9).

One independent determinant of the pCO_2 level is also the content of the volatile fatty acids in ruminal fluid. In the case of feeding according to maintenance requirements, the VFA sum total in rumen was 17.7...84.6 mmol/l. The correlation between the ruminal fluid VFA sum total and blood plasma pCO_2 has been presented in figure 10.

Blood ammonia content did not change significantly in connection with the increase in dry matter protein content in the feed ration. The daily average values of blood NH_4-content varied 1.53...1.93 mmol/l. The minimum and maximum values of ruminal NH_4 were 0.4 and 4.8 mmol/l. The blood NH_4-content daily average values were not different with rations used in the experiment (tab. 2, fig. 1,B). The

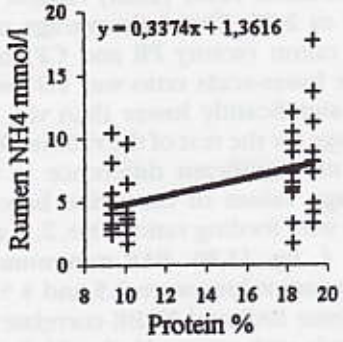

Figure 2. Dependence of rumen NH_4-content on protein content in DM

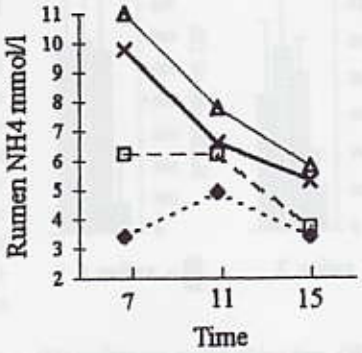

Figure 3. Daily dynamics of rumen NH_4-content by different rations

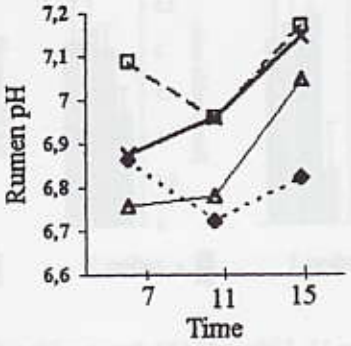

Figure 4. Daily dynamics of rumen pH by different rations

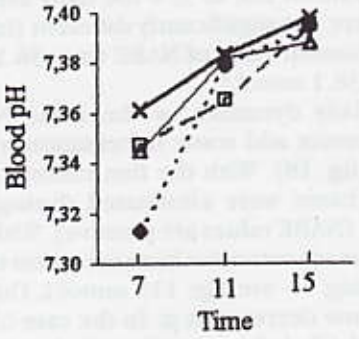

Figure 5. Daily dynamics of blood pH by different rations

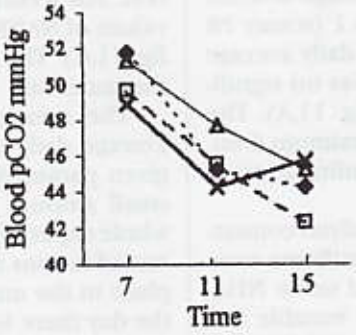

Figure 6. Daily dynamics of blood pCO_2 by different rations

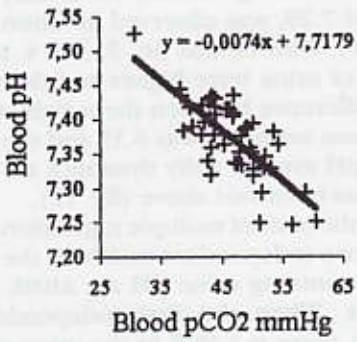

Figure 7. Dependence of blood pH on blood pCO_2

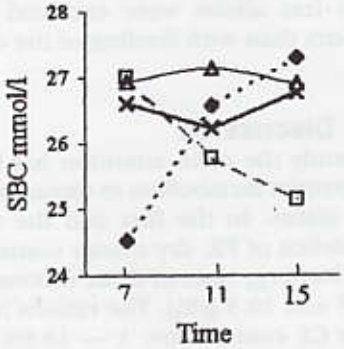

Figure 8. Daily dynamics of blood SBC by different rations

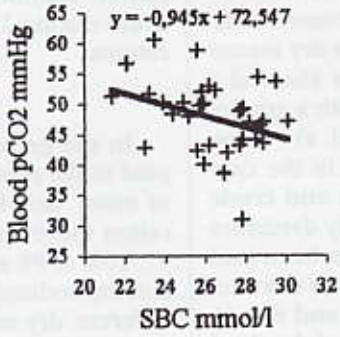

Figure 9. Dependence of blood pCO_2 on blood SBC-content

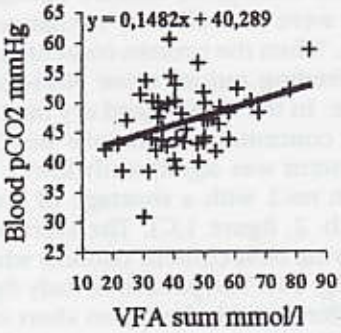

Figure 10. Dependence of blood pCO_2 on rumen VFA sum

Note for figures no. 3, 4, 5, 6 and 8:

◆ ..... ration 1 △ ——— ration 2

□ — — — ration 3 × ——— ration 4

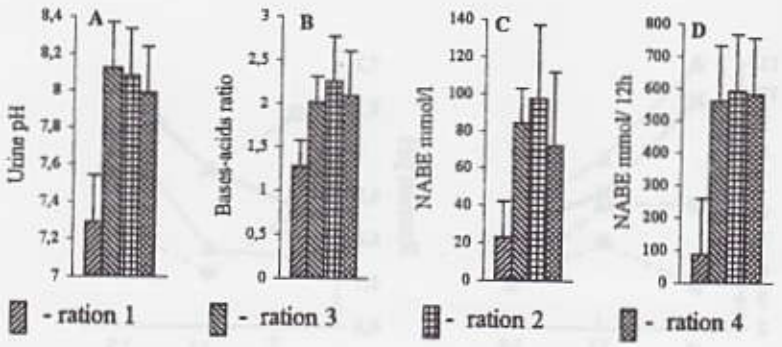

Figure 11. Urine pH (A), bases-acids ratio (B), net acids-bases excretion (C) daily average values and net acid-bases excretion in 12 hours (D) by different rations

blood NH_4-content was not in correlation with nutritive factors.

The **urine pH** daily average values were 7.29...8.12. A significantly lower daily average of urine pH, pH 7.29, was observed in ration no.1 (scanty PR and CF). With rations no. 2, 3 & 4, the daily average values of urine were higher and there was no significant difference between them (tab. 4, fig. 11,A). The minimum urine pH was 6.35 and the maximum 8.60. Urine pH average daily dynamics are confirmation of what has been said above (fig. 12).

On the basis of multiple regression analysis consisting of two independent variables, the significant ones for determining urine pH are ABNE and urine NH_4-content. When the first independent variable increases, there is a shift in the urine pH in the alkalic direction, in the case where the second independent variable increases, there is a shift in the acidic direction. These relations are characterized by the results of simple regression analysis (fig. 13 and 14).

The daily average values of **urine ammonia content** varied 3.77...12.21 mmol/l (tab. 2, fig. 1,C). The minimum value of this variable was 0.80 and the maximum value 33.63 mmol/l. Changes of urine NH4-content were contradictory to changes in rumen NH_4-content. When the protein content grew in dry matter of the feeding ration, urine NH4-content showed a decrease. In the case of feed dry matter with a greater protein concentration (rations no. 2 and 4) urine NH_4-content was significantly lower than in the case of ration no.1 with a shortage of protein and crude fibre (tab. 2, figure 1,C). The average daily dynamics of the urine NH_4-content confirm what has been said above (fig. 15). The pattern of daily dynamics is somewhat different with the ration short of PR and rich in CF (no. 3). The daily dynamics in the case of the third ration at 7 a.m. and 11 a.m. coincided with the daily dynamics of the urine NH_4-content registered by protein-rich rations. Urine NH_4-content depended on the feed ration protein amount and protein-energy ratio (PRg/MJ). While the values of these factors increased in urine, NH4-content decreased (fig. 16).

The daily average values of the **NH_4 elimated with urine during 12 hours** reached 28.4...73.7 mmol. The differences between rations of this indicator are not statistically significant (fig. 1,D). The minimum and maximum values were correspondingly 3.36 and 333.01 mmol.

The daily average values of the **urine bases-acids ratio (BAR)** ranged from 1.27 to 2.25. The daily average of the first ration (scanty PR and CF) of the urine bases-acids ratio was 1.27 which was significantly lower than the daily average for the rest of the rations. There was no significant difference in daily average values of the urine base-acid ratio with feeding rations no. 2, 3 and 4 (tab. 4, fig. 11,B). BAR minimum and maximum values were 0.5 and 4.5.

Urine BAR and NABE correlate very strongly with urine pH (fig. 14 & 17).

The daily average values of the **urine acids-bases net excretion (NABE)** varied within greater limits and ranged from +22.8 to +97.1 mmol/l.

The daily average values of NABE were significantly lower (22.8 mmol/l) with the first ration than with the rest. For feeding rations no. 2, 3, 4 the daily average values of NABE were not significantly different (tab. 4, fig. 11,C). The minimum value of NABE was –36.1 and the maximum +238.1 mmol/l.

The average daily dynamics of the urine NABE average daily dynamics add some information to the given parameter (fig. 18). With the first ration a very small amount of bases were eliminated during the whole experiment (NABE values are positive). With the rest of rations the most extensive base excretion takes place in the morning — average 115 mmol/l. During the day there is some decrease in it. In the case of the ration with PR and CF deficit, significantly less bases were eliminated at 7 a.m. and 3 p.m. than with other rations.

The daily average values of **acid-base net excretion with urine during 12 hours (NABE 12h)** expressed in mmol-s changed analogically to the previous indicator and ranged from +85.8 mmol to +592.4 mmol (tab. 4, fig. 11,D). In case of the first ration, significantly less alkalis were excreted with urine during 12 hours than with feeding of the other rations.

Discussion

In the present study the main attention has been paid to the role of protein metabolism in disturbances of cows' acid-base status. In the first and the third ration there was a deficit of PR: dry matter contained 9...10% of PR and PR-energy ratio in those rations was correspondingly 9.5 and 10.3 g/MJ. The rations had a different dry matter CF content: no. 1 — 18.6% and no. 3 — 27.6%. In the second and fourth ration there was an excess of protein — dry matter contained correspondingly 19.3% and 18.3% of PR and the PR-energy ratio was 17.5...18.9 g/MJ. In dry matter there was approximately the same amount of CF as in the previous rations — nos. 1 and 3 — correspondingly 19.0 and 28.0%.

When our experimental cattle were fed rations with a shortage of PR and CF, the daily average of pH of the ruminal fluid was the lowest. When the CF content was increased in the ration with a PR deficit, ruminal pH increased as well. This change is in accordance with data of literature (P. Nørgaard, 1993). In rations with

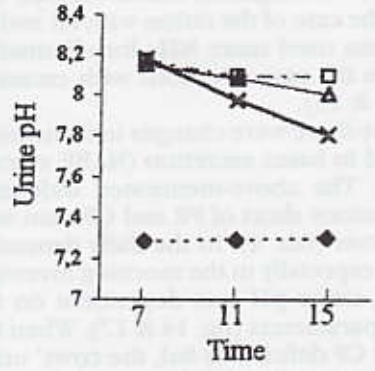

Figure 12. Daily dynamics of urine pH by different rations

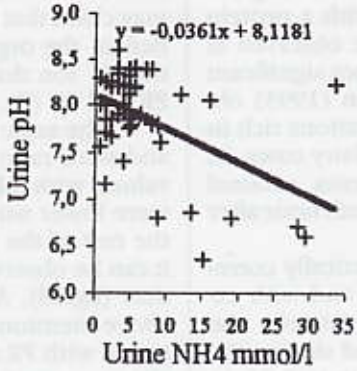

Figure 13. Dependence of urine pH on NH_4-content in urine

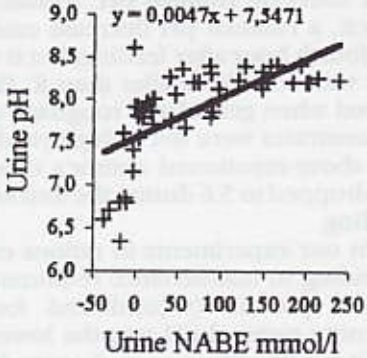

Figure 14. Dependence of urine pH on net acid-base excretion in urine

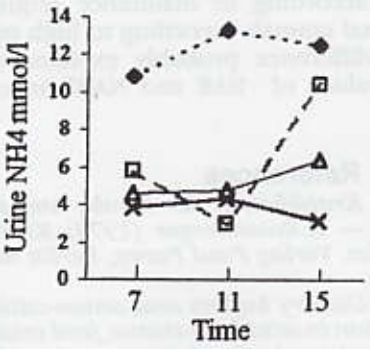

Figure 15. Daily dynamics of urine NH_4-content by different rations

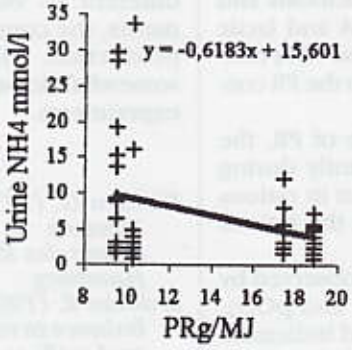

Figure 16. Dependence of urine NH_4-content on protein and energy ratio in DM

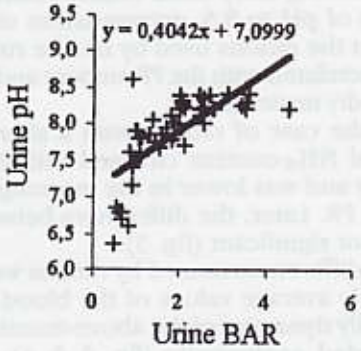

Figure 17. Dependence of urine pH on base.acid relatio in urine

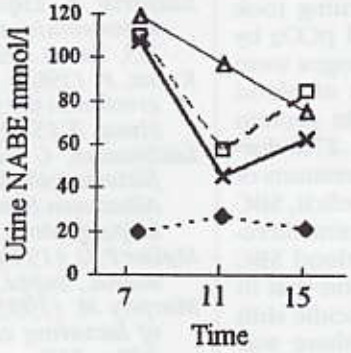

Figure 18. Daily dynamics of urine net acid-base excretion in urine

Note for figures no. 12, 15 and 18:

◆.....ration 1 △_____ ration 2

□ _ _ _ ration 3 ×_____ **ration 4**

excessive PR, the CF content variation had no significant effect on ruminal pH. In rations with a protein deficit, a ruminal pH decrease could be observed at the fourth hour after feeding, but it was not significant and was notably smaller than R. Erdman (1993) observed when granulated roughage and rations rich in concentrates were fed to high-yielding dairy cows. In the above-mentioned author's experiments, ruminal pH dropped to 5.6 during the fourth to sixth hour after feeding.

In our experiments in rations energetically corresponding to maintenance requirements and with excessive protein (second and fourth ration), the morning ruminal pH was the lowest and during the day it continually increased, extending up to 7.05. In our experiments the interval between evening and morning feeding was longer — 14 hours. In rations rich in protein, the after-feeding pH decrease probably took place later and the low point of pH remained in the morning feeding-time due to slower twenty-four-hour dynamics (fig. 4).

M. Murphy (1983) and P.D. Møller (1995) have claimed that feeding large amounts of concentrates to high-yielding dairy cattle causes ruminal acidosis and a drop of pH to 5.5, accumulation of VFA and lactic acid. In the rations used by us, the ruminal NH_4-content correlated with the PR amount and also the PR-content in dry matter (fig. 2).

In the case of rations with a shortage of PR, the ruminal NH_4-content changed insignificantly during the day and was lower in the morning than in rations rich in PR. Later, the differences between the rations were not significant (fig. 3).

No differences caused by rations were observed by the daily average values of the blood pH and pCO_2. The daily dynamics of the above-mentioned indicators proceeded analogically (fig. 5 & 6). Together with digestion, blood pH grew to some extent during the day at the same time as pCO_2 dropped. The average pCO_2 in the morning (ca 50 mm Hg) should be considered high. The afternoon pCO_2 level (ca 45 mmHg) is notably closer to the cows' physiological pCO_2 level. Changes in carbon dioxide partial pressure refer to the fact that in an acid-base status system, the compensation of the blood pH decrease in the morning took place through lungs with increasing blood pCO_2 by hypoventilation. At the same time SBC changes were also taken into account in the evaluation of blood acid-base status shifts. When rations rich in protein were consumed, SBC values changed 26.5...27.0 during the day. This refers to the greater regenerations of SBC in the kidneys. With the rations of PR-deficit, SBC daily dynamics varied more and had different directions (fig. 8). The negative correlation of blood SBC and pCO_2 (fig. 9) may lead to the conclusion that in the morning, in the case of the blood pH acidic shift when blood pCO_2 values had increased, there was relatively little SBC in the blood. A small amount of SBC means the existence of acidic shift and a large amount of SBC means alkalic shift. Consuming rations with excessive PR caused an increase of SBC-content by some mmol/l — thus alkalic shift of blood acid-base status.

When rations short of PR and CF were fed, the cows' urine pH daily average was lower than the determined values of the rest of rations. This fact is also observed by the pH daily dynamics (tab. 3, fig. 12). Thus, feeding the first ration caused significantly more acid urine excretion. The urine NH_4-content correlation with urine pH was negative. Based on this, one may claim that in the case of the ration with PR and CF deficit, the organism used more NH_4 for eliminating the H^+ ion than in the case of rations with excessive PR and CF (fig. 13 & 15).

At the same time there were changes in urine bases and acids ratio and in bases excretion (NABE average values with plus). The above-mentioned indicators were lower with rations short of PR and CF than with the rest of the rations (tab. 4). In the daily dynamics, it can be observed especially in the morning investigation (fig.18). Also, urine pH was dependent on the above-mentioned parameters (fig. 14 & 17). When the ration with PR and CF deficit was fed, the cows' urine NH_4 increased and BAR and NABE decreased. Changes like that refer to alimentary acidosis, according to G. Lachmann and M. Schäfer (1985).

In our experiments, when rations rich in PR and CF were fed, cows' urine acid-base status parameters changes were similar to urine NH_4, BAR and NABE changes in cows with alkalic feeding anamnesis investigated by the above-mentioned authors. The feeding levels of our and their experimental animals were different — ours according to maintance requirements, the compared animals according to high milk production. This difference probably explains the somewhat lower values of BAR and NABE in our experiments.

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About Causes Of Pre- And Early Postnatal Mortality In Calves And Pathological Changes Found In Them

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Abstract

Of 30 calves dissected and examined, of which 3 were aborted, 21 stillborn and 6 died after birth, 28 or 93.3% were infected with chlamydiae. Chlamydial inclusions in the affected calves were detected most frequently and in greatest numbers in the spinal cord (100%), medulla oblongata (96.4%), pons cerebri (95.5%) and the white matter of the cerebellum (80.8%). In 52.9% of the studied cases (17 calves) chlamydiosis was associated with viroses. In most cases pathological changes were localized in the digestive tract in the form of an acute catarrhal or hemorrhagic abomasitis and enteritis and degeneration of the liver.

Key words: Calves, chlamydiae, brain, spinal cord

Introduction

Causes of mortality in calves have been studied since 1987 (1). In 1988 research into the causes of loss of calves was carried out in 56 large dairy units situated in 14 counties. The number of milking cows kept in the large dairy units was 400 to 600 at that time. The total loss of calves was 9765, i.e. 15.4%, great differences (from 6.6% to 34.4%) occurring between the cattle sheds. Abortions formed 1.9% of the total loss, stillbirths 5.7%, 5% of born calves died and 3.5% of the calves were slaughtered in an emergency. According to the data of the State Veterinary Department, the loss of calves in the republic in 1987 was 47561, or 15.3%. Half of the losses were due to abortions, especially stillbirths, the other half was constituted by those who died or were slaughtered in an emergency. Similar losses also occurred in the republic in subsequent years.

Our earlier studies (2) have established chlamydiosis to be one of the causes responsible for losses in large dairy units: stillbirths and deaths and birth of unviable neonates. In part of the studied calves chlamydiosis was associated with viroses. This was also the case in one stillborn calf. All the calves investigated were kept in different large dairy units.

A. H. McNutt (3) is known to be first to have established chlamydial infection also in another mammals besides man. In 1940 he claimed that chlamydiae produce infectious encephalitis and meningitis only in young cattle and calves. Since that time the disease has been referred to as sporadic bovine encephalomyelitis (4).

Many subsequent investigators have also established the etiological role of chlamydiae in different species of domestic and wild animals besides calves (5,6,7), causing pneumoenterites, arthrites, conjunctivites, malformations, etc. beside encephalomyelitis.

O. Rasputina (8), Novosibirsk province, Russia, has studied the brains of calves ill with chlamydiosis in large dairy units, diagnosing encephalitis in 57% of calves in the case of intrauterine chlamydiosis and in 24% of calves in the case of postnatal chlamydiosis.

A. Rasputina's research inspired us to study the affection of different parts of the brain and spinal cord by chlamydiae in Estonian herds of cattle, first of all in large dairy units. We have studied chlamydiae induced pathological changes in their different organs, including the nervous system. The aim of our study was to ascertain causes of mortality of 1) aborted, stillborn calves and those who died immediately after birth, 2) pathological changes occuring in them, 3) localization of chlamydiae and their intensity in different parts of the calves' central nervous system.

Material and methods

The present research only involved the dissection and study of the carcasses of aborted and stillborn calves and of those who died immediately after birth for evidence of intrauterine infection and subsequent pathological changes. The total of 30 calves were dissected in 1994 to 1995. 3 of them were aborted (in the 5th to 7th month of pregnancy), 21 were stillborn (part of them died during parturition, 6 calves born lacked vitality and died immediately after birth — 0 days of age. The dissected calves originated from 14 different large dairy units of 6 counties, most of them (20) from 8 large dairy units of Tartu county and 2 small farms' cattlesheds.

In dissected calves the occurrence and nature of pathological changes was ascertained. The pathological material necessary for histological examination was fixed in formalin, introduced in paraffin and sections were stained with hematoxylin-eosine and hematoxylin-picrofuchsine. To clarify the localization of chlamydiae and their intensity (numbers) the brain was examined at nine different sites (fig. 1): I, II, III

cortex of the cerebrum, IV medulla oblongata, V cortex of the cerebellum, VI white matter of the cerebellum, VII callosum, VIII hippocampus, IX pons. Parallel with the former in some of the calves dissected also the spinal cord was examined: X the cervical part, XI the thoracic part and XII the lumber part of the spinal cord.

Our earlier studies (9) have confirmed the modified Stamp method to be the most suitable one for the detection of chlamydiae (resp. chlamydial inclusions) in the smears made of pathological material (Bortnichuk, 1991). In the stained smears the frequency of occurrence of chlamydiae (intensity) was estimated according to 4-point-scale:

1 (+):1 to 2 chlamydiae in five observation areas;
2 (++):5 to 10 chlamydiae in most of the areas;
3 (+++):15 to 25 chlamydiae in each area;
4 (++++): a great number of chlamydiae per area. Althougth this system of estimation is inexact and subjective, it still gives a relative survey of the occurrence and intensity of chlamydiae in different parts of the brain. Parallel with the Stamp staining the indirect immunofluorescence method was applied in the examination of the brain and spinal cord of 20 calves. The examination was carried out at the laboratory of the Hospital of Infectious Diseases of Tartu University. As *Chlamydia trachomatis* and *Chlamydia psittaci* have common group specific antigens, diagnostic antigens for man were used to detect chlamydiae (producer Biotechnologicheskaya compania Bioservice, Moscow).

Pathological material from 13 calves was also bacteriologically examined. The material of 17 calves was examined for virus diseases of a more common spread (AD, Rs, IBR/IPV, VD/MD, rotavirosis). The examinations were carried out at the bacteriological and virological laboratories of the Estonian Agricultural University employing standard methods.

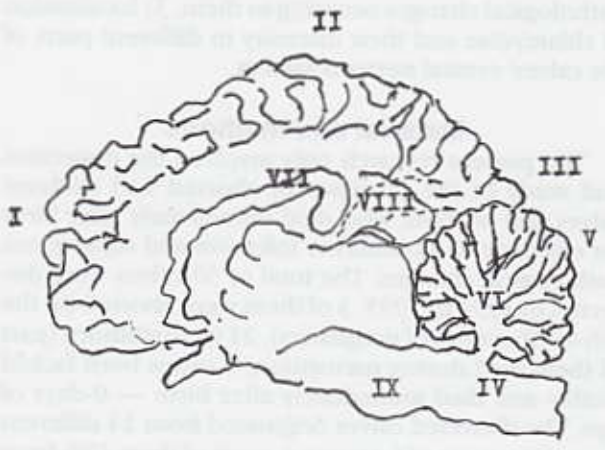

Figure 1. *Scheme of the brain of the calf.*
Sites of sampling:
I, II, III – cortex cerebri
IV – medulla oblongata
V – gray matter of the cerebellum
VI – white matter of the cerebellum
VII – callosum
VIII – hippocampus
IX – pons

Results

Tables 1 and 2 show that chlamydial inclusions were found in only one (No. 148) out of three aborted calves examined (the calf was aborted in the 7th month of pregnancy). Chlamydiae were detected in all 21 stillborn calves (100%). Chlamydial inclusions were also found in all 6 calves who died immidiately after birth (100%).

It is seen from tables 2 and 3 that the organ most frequently affected by chlamydiae was the spinal cord, where they were also most numerous. They were detected in the cervical, thoracic as well as lumbar part of the spinal cord, in which regions chlamydial inclusions were found in all the calves affected by chlamydiae (100%). In 27 calves (96.4%) chlamydiae were found in the medulla oblongata, in 21 of them (77.8%) chlamydiae were numerous in the brain smear (++++). Of 22 calves examined in 21 (95.5%) chlamydiae were present in pons cerebri, in 13 of them (61.9%) chalmydial infection was very intensive (++++). The presence of chlamydiae in the white matter of the cerebellum was detected in 21 out of 26 calves examined (80.8%), chlamydial inclusions having been found in great numbers (++++) in 9 of them (42.9%). Chlamydial affection was less than 50% in the cortex of the cerebellum, where they were found in 12 out of 25 calves examined (48%), the intensity of infection being weaker too (+, ++). In 8 calves (33.3%) out of 24 examined, chlamydiae were found in the smear made of the hippocampus whereas in 7 of them the intensity was weak (+). Chlamydiae were found less frequently in the cortex of the hemispheres as compared with the other parts of the brain (in 12.5 to 22.2% calves examined), the intensity of chlamydiae present also being weak in most cases (+, ++). In 15 calves the presence of chlamydiae in different parts of the brain was also examined by the method of immunofluorescence. Tables 1 and 2 show that of 15 calves examined by the method of immunofluorescence in whom chlamydiae were detected by microscopic study, intracellular fluorescent chlamydial inclusions were found in 14 (93.3%). In the medulla oblongata of one unviable neonate chlamydial inclusions (fig. 2) were also detected by the electron microscopic study.

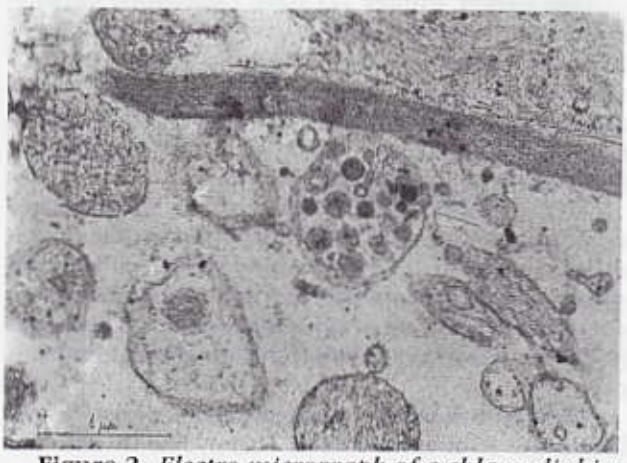

Figure 2. *Electro micrograph of a chlamydial inclusion (arrow) in the medulla oblongata of an unviable newborn calf, killed when 4 hours old. The bar equals 1 μm (Orig.).*

Research for viral diseases

For the diagnostic purpose, the pathological material of 17 calves dissected was examined not only for the detection of chlamydiae, but also for viral discases. Tables 1 and 2 show that of the calves examined 2 had been aborted (Nos. 119, 131), 12 stillborn, (Nos. 118, 208, 227, 124, 132, 211, 125, 231, 238, 228, 196, 243), and 3 calves (Nos. 157, 242, 241) died immediately after birth (0 days of age). The research proved positive in the case of 9 calves (52,9%), 7 of whom having been stillborn and 2 having died immediately after birth. The calves originated from 7 large dairy units in 3 counties (Table 2). It is evident from tables 1 and 2 that virus infections could not be ascertained in the aborted calves. In the case of the 12 stillborn calves, we succeeded in ascertaining virus infection in 7 calves (58.3%): in one calf (No. 124) VD/MD was detected, AD in one calf (No. 243), rotavirus infection in 4 calves (Nos. 211, 231, 228, 194). All the calves originated from different large dairy units of 2 counties. One calf had diverse infection AD+RS. Of the 3 calves who died immediately after birth virus disease agents were diagnosed in two (66.7%), VD/MD in one of them (No. 242), AD in the other (No. 241), the calves originating from different large dairy units of the same county. Table 2 shows that calves examined for virus infection originated from 9 different dairy units situated in 2 counties, chlamydiae having been detected in the calves' pathological material in all the units mentioned. Of these large dairy units only in 2 (cattle-sheds 1 and 4) situated in the same county were agents of virus diseases not found in the pathological material of the calves examined in 1994 and 1995. Of the seven units where viral diseases were diagnosed, rotavirosis was in three, AD in two and VD/MD in one. In one cattle-shed (No. 3) several virus infections were established in the calves' pathological material: AD+RS in one stillborn calf (No. 124), rotavirus infection in the other stillborn animal (No. 211) and VD/MD in one calf who died immediately after birth (No. 242).

It became clear from the results of the research that of the 17 calves examined, chlamydiosis was associated with viral diseases in 9 (52.9%), whereas of 9 large units under examination, virus infections were present in 7.

Bacteriological research

Bacteriological investigation (tables 1 and 2) was carried out on 15 calves dissected. The pathological material of two aborted, twelve stillborn and one calf who died immedialtely after birth (0 days old) was bacteriologically examined. The results of the research were negative in all the calves investigated.

Pathologico-anatomical research

Dissection results of aborted calves

Table 3 shows that in three calves, aborted from the 5th to the 7th month of pregnancy, degenerative changes were found in the liver. In the oldest of them chlamydial inclusions were detected in the cortex of the hemisphere of the brain. The bacteriological and virological findings of two calves examined proved negative.

Dissection findings of stillborn calves

Most of these calves were born dead at the end of pregnancy, part of them were premature (born later than the 7th month of pregnancy). One calf was malformed (*perosoma elumbis*).

Pathological-anatomical changes were observed in all 21 stillborn calves (100%). In three calves pathological changes occurred in the abomasum as an acute catarrhal inflammation. Eleven calves had enteritis (52.4%), seven of them an acute catarrhal, three partly hemorrhagic inflammation. One calf had subacute catarrhal enteritis. In six calves inflammation was localized only in the small intestines, in the remaining 5 calves simultaneously also in the large intestines.

Pathological changes were found in the liver in 15 calves (71.4%), of whom degenerative changes had developed in 14 (87.5%), one of them having cirrhosis simultaneously, one necrosis and one revealing characteristics of congestive hyperemia. In one calf passive hyperemia was observed in the liver.

Kidney pathology manifested itself in six calves (28.6%), in four of them as degeneration, in one as hyperemia and in one as characteristics of inflammation.

Heart pathology was observed in nine calves (42.9%): three calves had the degeneration of the cardiac muscle, one had heart dilatation, one calf showed characteristics of pericarditis, in four calves subepicardiac hemorrhages were found.

Pathological changes in the spleen were found in four calves 19.0%). In two of them the spleen was swollen, one had hemorrhagic infarction of the spleen, and in the spleen of one calf subcapsular petechia were seen.

In all stillborn calves (or in calves who died during partutition) the lungs were either entirely or partly atelectated, in two calves characteristics of hyperemia were noted.

Charecteristics of hyperemia of the brain and meninges were observed in six calves (28,6%).

Hydropsy of the thoracic and abdominal cavities was found in one calf, hemorrhages in the thymus were present in one calf, the thyroid gland had enlarged in seven calves, the heaviest of the glands weighing 639 grammes.

Dissection findings of calves who died immediately after birth (0 days of age)

Pathological changes were found in all the six calves who died immediately after birth (100%).

An acute catarrhal abomasitis was observed in one calf (16.7%), an acute catarrhal inflammation of the small and large intestines was seen in two calves (33.3%), in one of them it was hemorrhagic in spots.

Pathological changes in the liver were observed in all the six calves dissected (100%): degenerative changes were present in five, chirrosis in one of them. In one calf charecteristics of passive hyperemia were found.

In the kidneys as well as in the spleen pathological changes were not observed.

Pathology of the heart was found in three calves (50%): in one of them characteristics of degeneration of the cardiac muscle occurred, in two calves the heart was dilated.

Pathology of the lungs was observed in three calves: charecteristics of an acute catarrhal inflammation in association with the manifestation of an acute catarrhal

Table 1. *Results of laboratory examinations of dissected calves.*

Method of investigation	Calves			In all
	Ab*1	Sb*2	Nb*3	
Microscopic investigations for chlamydes	3	21	6	30
of them positive	1	21	6	28
of them negative	2	0	0	2
Investigations for chlamydes by the indirect immunofluorescence method	11	11	4	15
of them positive		10	4	14
of them negative		1	0	1
Virological investigations	2	12	3	17
of them positive	0	7	2	9
of them negative	2	5	1	8
Bacteriological investigations	2	12	1	15
of them positive	0	0	0	0
of them negative	2	12	1	15

*Comment: *1 – aborted; *2 – stillborn; *3 – newborn (0 days of age old)*

Table 2. *Results of laboratory examinations of aborted (Ab), stillborn (Sb) and newborn (NB - 0 day of age) calves.*

No of calves	Cowshed county	Ab Sb Nb	Examination of chlamydes: microscopical: brain I	II	III	IV	V	VI	VII	VIII	spinal cord IX	X	XI	XII	by indirect immuno-fluorescence method: examined material	result	Bacter. examin.	Virol examin.
119	1, Tartu	Ab		0													negat.	negat.
151		Ab	0	0	0	0	0	0	0	0	0						negat.	negat.
118		Sb	0	0	0	4	0	0	0	0	4						negat.	negat.
208		Sb	0	0	0	4	0	3	0	0	4	4	4	4			negat.	negat.
227		Sb	0	0	0	4	0	1	0	1	3	4		4	IV, X, XII	negat.	negat.	negat.
232		Nb	0	0	0	4	0	4	0	1	2	4			V,IX	posit.		
124	2, Tartu	Sb	0	0	0	4	0	1	0	1	2						negat.	VD/MD
157		Nb	0	0	0	3	1	2	0	0	1						negat.	negat.
132	3,Tartu	Sb	1	0	0	1	0	0	0	0							negat.	AD/+RS
211		Sb	1	0	0	4	1	3	1	0	3	4	4	4	IV	posit.	negat.	Rota-
242		Nb	0	0	0	4	1	4	0	0	4	4	4	4	IX	posit.		AD,VD/MD
125	4, Tartu	Sb	0	0	0	4	0	0	0	0	4						negat.	negat.
231	5,Tartu	Sb	0	0	0	4	0	1							IV, IX	posit.	negat.	Rota-
238	6, Tartu	Sb	0	1	0	4	2	4	2	1	3			4				
228	7, Tartu	Sb	0	0	0	4	2	4	0	0	4	4		4	IV, X, XII	posit.	negat.	Rota-
229		Sb	0	1	1	4	1	4	0	1	4	4			VI, X	posit.	negat.	
241	8, Tartu	Nb	0	0	0	4	1	2	0	0	4	4		4	IV	posit.		AD
221	9, Tartu	Sb				3		4										
184		Nb		0		4	0	1	1	1	4	4	4	4	XII	posit.		
156	10, Tartu	Sb	0	0	0	3	0	0	0	0								
134	11, Tartu	Sb				1												
148	12, Tartu	Ab	0			1												
151	13, Tartu	Sb		1													negat.	Rota-
240	14, Tartu	Sb	0	0	0	4	0	1	0	0	4	4	4	4	IV	posit.	negat.	negat.
194	15, Paide	Sb	0	0	0	4	1	1	0	0	3	4						
		Sb	0	0	1	4	2	4	1	0	4	4	4	4	IV	posit.		
		Sb	2	1	1	4	1	4	0	1	4	4	4	4	XII	posit.		
		Sb	1	1	0	4	2	4	1	3	4	4	4	4	XI	posit.		
		Nb	0	0	0	4	1	2	1	0	1	4	4	4				
243	16, Paide	Sb	0	1	0	4	0	1	0	0	4				IX	posit.		AD
Total		30	25	27	24	28	25	26	24	24	22	15	9	13			13	17
posit. of them			4	6	3	27	12	21	6	8	21	15	9	13			0	9
%			16	22,2	12,5	96,4	48	80,8	25	33,3	95,5	100	100	100			0	52,9
negat. of them			21	21	21	1	13	5	18	16	1	0	0	0			13	8
%			84	77,8	87,5	3,6	52	19,2	75	66,7	4,5	0	0	0			100	47,1

Comment: I, II, III – cortex cerebri; IV – oblongata; V, VI – cerebellum;VII – callosum; VIII – hippocampus; IX – pons; X – cervical; XI – thoracis; XII – lumbar part of spinal cord.

Table 3. *Affection by chlamydes of the brain and spinal cord of the aborted, stillborn and newborn calves.*

Samples examined	*Cortex cerebri*						*Medulla obl.*		*Cerebellum*			
	I		II		III		IV		grey matter V		white matter VI	
	No	%	No	%	No	%	No	%	No	%	No	%
in all	**25**		**27**		**24**		**28**		**25**		**26**	
negat. of them	21	84	21	77,8	21	87,5	1	3,6	13	52	5	19,2
posit. of them	4	16	6	22,2	3	12,5	27	96,4	12	48	21	80,8
of them												
+ (1)	3	75	6	100	3	100	3	11,1	8	66,7	7	33,3
++(2)	1	25	0		0		0		4	33,3	3	14,3
+++(3)	0		0		0		3	11,1	0		2	9,5
++++(4)	0		0		0		21	77,8	0		9	42,9

Samples examined	*Callosum*		*Hippocampus*		*Pons*		Spinal cord					
	VII		VIII		IX		X		XI		XII	
	No	%	No	%	No	%	No	%	No	%	No	%
in all	**24**		**24**		**22**		**15**		**9**		**13**	
negat. of them	18	75	16	66,7	1	4,5	0	0	0	0	0	0
posit. of them	6	25	8	33,3	21	95,5	15	100	9	100	13	100
of them												
+ (1)	5	83,3	7	87,5	1	4,8	0		0		0	
++(2)	1	16,7	0		3	14,3	0		0		0	
+++(3)	0		1	12,5	4	19,0	0		0		0	
++++(4)	0		0		13	61,9	15	100,0	9	100,0	13	100,0

Table 4. *The results of dissection of aborted (Ab), stillborn (Sb) and newborn (0 days old) calves.*

Calves age	No	Abomasum inflammation			Intestine inflammation				Liver				Kidney				Heart				Spleen				Lungs				Changes in some other places			
		in all	acute cat.	subacute cat.	in all	acute cat.	subacute cat.	hemorrhagic	pat. changes in all	degeneration	venous hyperemia	hemorrhages	pat. changes in all	degeneration	hyperemia	inflammation	pat. changes in all	degeneration	dilatation	hemorrhages	pericarditis	pat. changes in all	swelling	hemorrhages	infarction hemorrh.	path. changes in all	pneumonia cat.	hyperemia	atelectasia	acute cat. rhinitis	ascites, hydrothorax	hemorrhages in the thymus	hyperemia of brain and mening.	enlargement of thyroid gland	malformation
Ab	3																																	
Sb	2	3	3		11	7	1	3	15	14**	1		6	4	1	1	9	3	1	4	1	4	2	1	1	2	2							
Nb	6	1	1		2	2			6	5***	1						3	1	2							3	1	2	2	1	1	1	6	7	1
		4	4		13	9	1	3	24	22	2		6	4	1	1	12	4	3	4	1	4	2	1	1	5	1	2	2	1	1	1	7	10	1

Comment:

* *Inflammation was localized in small intestine only in six calves;*

** *Degeneration alongside of cirrhosis in one calf, degeneration parallel with necrosis in one, degeneration alongside of venous hyperemia in one;*

*** *Degeneration with cirrhosis in one calf.*

rhinitis in one calf and characteristics of atelectasis in two.

The thyroid gland had enlarged in 3 calves, weighing 750 grammes in one of them.

Summary and conclusions

On the basis of our research we reached the conclusion that in the Estonian large dairy units a cause of stillbirths and death of calves immediately after birth is chlamydiosis, often being associated with viroses. Of 30 calves dissected chlamydiae were not found only in two, both having been aborted, one in the sixth, the other in the seventh month of pregnancy.

Of 17 calves, examined for virus diseases, in whom chlamydiae were detected, virosis was diagnosed in 9 (52.9%), rotavirosis in four of them, AD in two, VD/MD in one and combined virosis in two: one had AD/RS, the other AD+VD/MD.

In all the calves dissected, pathological changes were observed localizing in the digestive tract in most cases: an acute catarrhal abomasitis occurred in 4 calves (13.3%), an acute catarrhal or hemorrhagic enteritis in 12 (40.0%). Degeneration of the liver was observed in 19 calves (63.3%).

Pathological changes in the calves dissected were found also in other organ systems: heart pathology in 12 calves (40.0%), kidney pathology in 6 (20%), spleen pathology in 4 (13.3%). In the heart the degeneration of the cardiac muscle, cardiac dilatation and subepicardiac hemorrhages were observed. In the kidneys characteristics of degeneration were seen. Spleen pathology was evident in two calves as an enlargement of the organ. One calf had subcapsular hemorrhages. In one calf hemorrhagic infarctum was observed. Lungs pathology was observed in 5 calves (16.7%), of which two stillborn calves revealed characteristics of hyperemia. Of three calves who were born alive, one showed an acute catarrhal inflammation, two had partial atelectasis. Enlargement of the thyroid gland was recorded in 10 calves (55.5%).

Pathology of the central nervous system was observed in 7 calves (23.3%) in the form of hyperemia of the brain and meninges, although chlamydiae in the brain were detected in the spinal cord (in the cervical, thoracic and lumbar part) where they were found in all the calves examined. In the spinal cord chlamydiae were encountered in great numbers. In the brain it was the medulla oblongata in which chlamydiae occurred most frequently (96.4% of the calves examined). Also in the pons (95.5%), the numbers of chlamydiae was very large. In the matter of the cerebellum, chlamydiae were detected in 21 calves (80.8%), in the gray matter of the cerebellum only in 12 (48.0%). In the white matter of the cerebellum, the occurrence of chlamydiae was found to be very abundant in most cases. In the grey matter numbers were encountered in most cases. In fewer than 50% of the calves examined the hippocampus was affected by chlamydiae (33.3%), callosum (25%), cortex of the brain (12.5 to 16%). In these parts of the brain, chlamydiae occurred in small numbers in most cases.

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Ultrasound Guided Puncture Of Follicles In Heifers

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Abstract

The aims of the present study were to evaluate the function and the practicality of a modified device for ultrasound guided puncture of follicles and oocyte aspiration and to determine possible traumatic effects of long-term repeated oocyte aspirations on the genital tract in heifers. By using this modified device, puncture of follicles and aspiration of oocytes could be performed by one operator. During a period of 16 weeks the aspiration of oocytes in two heifers resulted in an overall recovery rate of 52.2%. Morphological examination of the genital tracts and ovaries post-mortem revealed a haematoma in one heifer and the presence of minor fibrous adhesions in another at the puncture site of the vaginal wall. Development of connective tissue was observed in one out of four ovaries.

Key words: ultrasonography, ovum pick up, modified device, heifers

Introduction

The recovery of oocytes from the ovaries of slaughtered animals has been the most common source of ova for *in vitro* fertilization. However, this source represents a non-repeatable approach and obtained embryos are mostly of less genetic interest for breeding programs (Pieterse et al., 1991, Kruip et al., 1994, Bols et al., 1995, Gibbons et al., 1995). Laparoscopic follicular oocyte aspiration combined with ultrasonography also has limitations due to scar tissue formation and adhesions at the operation site and in the ovaries (Pieterse et al., 1991, Gibbons et al., 1994, Looney et al., 1994, Bungartz et al., 1995, Stubbings & Walton, 1995). The ultrasound guided transvaginal follicular aspiration has been developed as an alternative method for obtaining oocytes from live animals (Pieterse et al., 1988). The method allows collection of oocytes with high degree of repeatability from animals of known genetic merit at any stage of the estrous cycle. This can be done with or without stimulation of follicular growth and from follicles at different stage of development over a period of several months (Pieterse et al., 1988, Pieterse et al., 1991,Walton et al., 1993, Bergfelt et al., 1994, Kruip et al., 1994, Looney et al., 1994, Vos et al., 1994, Pavasuthipaisit et al., 1995, Paul et al., 1995, Brogliatti et al., 1995, Bungartz et al., 1995, Dolman et al., 1995, Gibbons et al., 1995). The technique is more rapid and less traumatic for animals than aspiration of oocytes by laparoscopy (Pieterse et al., 1988, Pieterse et al., 1991, Bols et al., 1995, Stubbings & Walton, 1995). The efficiency of the ultrasonically guided puncture of follicles varies considerably and the recovery rate of oocytes depends on several factors, including the type of equipment used for aspiration (Pieterse et al., 1988, Simon et al., 1993, Scott et al., 1994, Vos et al., 1994, Bols et al., 1995, Lansbergen et al., 1995).

The aims of this study were to evaluate the function and the practicality of a technically modified device for ultrasound guided puncture of follicles and oocyte aspiration and to determine possible traumatic effects on the genital tract after long-term repeated oocyte aspirations in heifers.

Materials and methods

Animals

Two virgin heifers of the Swedish Red and White breed (13 and 14 months of age) were used in the experiment. The heifers were housed indoors in individual pens at the barn of the Department of Obstetrics and Gynaecology (Swedish University of Agricultural Sciences) and fed with good quality hay and concentrate, plus mineral supplement, according to Swedish standards.

The collection device

An ultrasound sector scanner (Scanner 250, Pie Medical, The Netherlands), with an endovaginal multiple angle transducer (Pie Medical, 5.0—7.5 MHz) was used. The transducer was inserted into a specially made stainless steel holder (length 60 cm, inner diameter 32.1 mm and outer diameter 38 mm) equipped with a ventrally positioned single lumen needle guide. (Figure 1.) The needle system for puncture of follicles consisted of two parts, a 58 cm long single stainless steel tube (inner diameter 1.2 mm and outer diameter 1.8 mm) and a 5 cm long disposable needle (inner diameter 0.8 mm and outer diameter1.1 mm). The puncture needle was attached in the end of the needle guide. The transducer and needle guidance holder were equipped with a handgrip (13.5 x 3.0 x

4.5 cm) which was designed to allow the movement of the puncture needle forth and back by a manually activated trigger, inserted in the handgrip. Before use, a guidline for the puncture needle was marked on the screen by testing the unit in water. The handgrip allowed the operator to manipulate and to fix the unit in the required position inside the vagina and, without assistance, to insert the needle through the vaginal wall into the visualized follicles. For aspiration of follicular fluid and oocytes the distal end of the steel tube was connected by silastic tubing (inner diameter 1.0 mm and outer diameter 2.0 mm, Corning Corp, USA) to a heparinized blood collection test tube (Venoject,Terumo Europe N.V., Belgium) with a small volume of phosphate buffered saline (PBS).The tube was through another silastic tubing connected to an electric suction pump (De Vilbiss Co,USA). Before being used, the steel tube and silastic tubing were rinsed with PBS plus heparin to prevent coagulation of the follicular fluid.

Collection of oocytes

The heifers were restrained in a crate in a standing position and sedated intravenously with Domosedan 1mg/100kg bodyweight (Orion Corp., Finland) to minimize their movement during aspiration. Epidural anesthesia was performed by administration of 4 ml Xylocain adrenalin (Astra Läkemedel, Sweden) to prevent rectal contractions. After evacuation of faeces from the rectum and thorough cleaning of the vulva and perineum, the aspiration device, covered with a latex cover containing contact gel, was inserted into the vagina and placed on the left or right side of the external os of the cervix. The ovary subjected to aspiration was positioned by manipulation per rectum on to the head of the transducer so that the follicle was transected by the biopsy line on the monitor.The needle was thereafter pushed through the vaginal wall into the follicle. Continuous suction was started as soon as the needle tip was seen in the follicle. After the follicle had collapsed, the needle was withdrawn and the next follicle was subjected to aspiration. At each collection session follicles larger than 3 mm in diameter were punctured. When aspirations were finished, the needle was removed from the holder and flushed with PBS plus heparin into the same collecting tube. Oocytes were recovered from the follicular fluid under a stereo microscope (Wild, M-8, Switzerland).

Morphological examinations post mortem

A week after the last aspiration the heifers were slaughtered and their genital tracts and ovaries subjected to gross examination. The ovaries were fixed in 10% formaline, and routinely embedded in paraffin. Paraffin wax sections (5 µm) were stained with haematoxylin and eosin to aid microscopic evaluation of possible ovarian aberrations after puncture.

Results

Transvaginal oocyte recovery was succesfully performed with our modified aspiration device.The instrument could easily be inserted into the vagina and the ovary pulled back close to the tip of the device. Manipulations, fixing of the device inside the vagina and the puncture of follicles were easily performed by the single operator. One or two collections per week and heifer were performed.

During the first 6 weeks the recovery rate of oocytes (percentage of oocytes recovered out of follicles punctured) was 36.4% (4/11). Following the next 6 weeks a recovery rate of 41.4% (8/18) was achieved and after the last 4 weeks the recovery rate reached 73.7% (14/19). In total the aspiration of oocytes during 16 weeks resulted in a recovery rate of 52.2% (26/48).

Gross examination of the genital tracts following slaughter one week after last aspiration revealed a haematoma on the cranial side of vagina in one heifer and, in the other, minor fibrous adhesions at the site of puncture of the vaginal wall (Figure 2). Histological examination of ovaries revealed an increased amount of connective tissue in one of the four ovaries (Figure 3).

Discussion

In the present study attention was paid to technical aspects of oocyte aspirations with a newly modified device for ultrasound guided follicular puncture. The major difference of the present device compared to other instruments which have been presented (Pieterse et al., 1988,Gibbons et al., 1994, Looney et al., 1994, Bols et al., 1995) is the possibility for the operator to control the movement of the aspiration needle.

In most other instruments presented, the puncture of the follicles is performed by an assistant who manually pushes the needle into the follicles (Pieterse et al., 1988, Gibbons et al., 1994, Scott et al., 1994, Bungartz et al., 1995). It is advantageous if the same person controls both the direction of the probe and the puncture of the follicles. A second advantage with the present unit is that disposable needles are used which are simply changed after each puncture session.

In the experiment, follicles larger 3 mm in diameter were punctured only in two heifers. This resulted in a limited total number of follicles punctured and oocytes aspirated. Also the person performing the puncture of follicles had no experience of the technique before the start of the experiment. Our results show, however, an increasing recovery rate by around 100% during 16 weeks ending up with final recovery rate of 74% which is comparable with other reports(Looney et al., 1994, Bungartz et al., 1995).The post mortem examination of the genital tracts after the last puncture session during 16 weeks showed only minor traumatic changes which are in agreement with other reports (Gibbons et al.,1994). It is unlikely that the morphological changes found in the two heifers in the present experiment would have influenced their future fertility.This assumption is confirmed by a study made by Metthews et al. (1995) where all 31 heifers subjected to repeated ovum pick up were inseminated after the end of the sessions and all heifers became pregnant within 68 days of the last ovum pick up session. In conclusion, our results show that the hereby presented device for ultrasound guided ovum pick up enables a single operator to control both the ultrasound transducer and puncture needle without causing any major trauma to the genital tract.

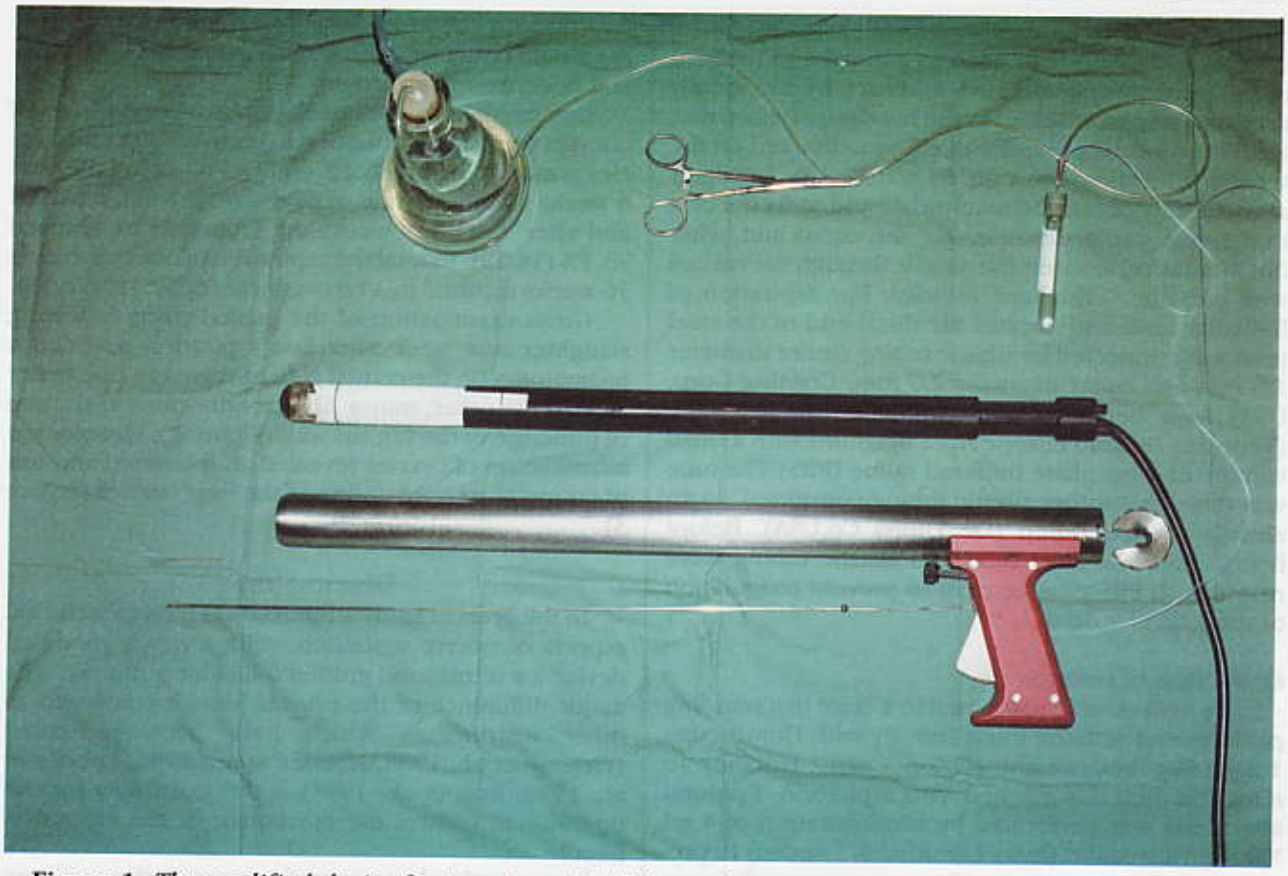

Figure 1. *The modified device for puncture of follicles and oocyte aspiration with the transducer holder and needle guide (see details in the text).*

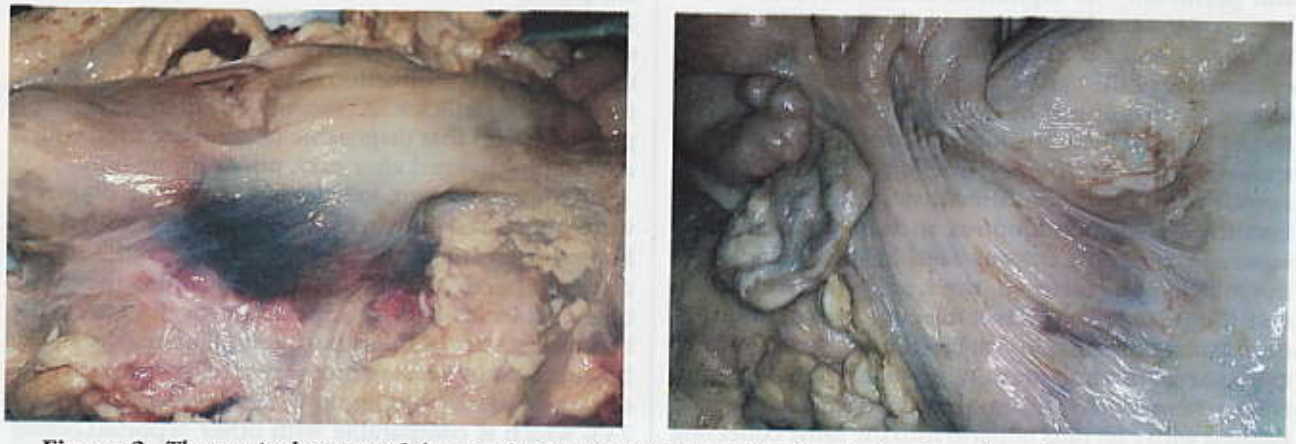

Figure 2. *The genital tracts of the two heifers* (**A;B**) *one week after last aspiration of oocytes.* **A.** *Haematoma at the cranial site of the vaginal wall;* **B.** *Fibrous adhesions at the site of puncture of vaginal wall.*

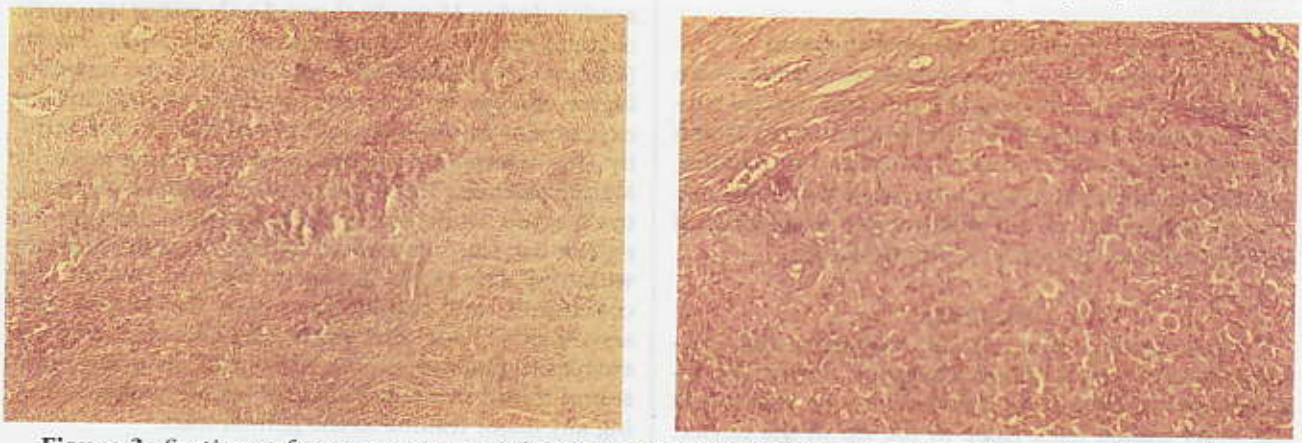

Figure 3. *Sections of an ovary one week after the last aspiration of oocytes (haematoxylin and eosin, magnification 32 X);* **a.** *Increased amount of connective tissue;* **b.** *The presence of a functional corpus luteum in the same ovary.*

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Evaluation Of The Corpus Luteum Activity In Synchronized Recipients On The Day Of Embryo Transfer

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Abstract

The present investigation was conducted to evaluate by rectal palpation the functional activity of corpora lutea formed in synchronized recipients on the day of embryo transfer and to compare the accuracy with blood serum progesterone radioimmunoassay. On the day of embryo transfer the majority (91.3%) of recipients synchronized by cloprostenol had corpora lutea evaluated by palpation as very good -39.4% and good — 36.8% (high functional activity) and as poor — 15.1% (low functional activity). In 4.3% cystic ovaries were diagnosed and in 4.4% ovaries did not contain any morphological structures. The high progesterone concentrations (≥2.0 ng/ml) have been found by blood serum RIA in 61.8% of recipients, in 31.3% progesterone was low and varied from 0.21 to 1.96 ng/ml. In the remaining 6.9% of recipients no luteal activity was found. The predictive value for the determination of the high luteal activity by palpation was 77.8%, sensitivity 86.4% and specificity 50%. More incorrect diagnosis have been made by palpation to predict low luteal activity. The predictive value was 64.5%, sensitivity 50.0% and specificity 86.4%. In the presence of the progesterone ≥2.0 ng/ml pregnancy rate of recipients was 23.9% higher (P<0.05) than in recipients with lower progesterone levels (<2.0 ng/ml).

Key words: recipients, corpus luteum, rectal palpation, progesterone, pregnancy

Introduction

A correct evaluation of the corpus luteum is essential for the determination of recipients' suitability for embryo transfer (Foote,1988, Broadbent et al., 1991). Following ovulation a corpus luteum develops from thecal and granulosal cells of follicle. As it becomes vascularized largely owing to the increase in size of luteal cells the corpus luteum increases in weight (Baird,1992, Garverick et al.,1992, Wiltbank & Niswender,1992) and reaches its maximum size on 8—10 days of estrous cycle (Rajamahendran et al.,1989, Ribadu et al.,1992), indicating high correlation between mature corpus luteum diameter and progesterone concentration in periferal blood (Seguin et al.,1974, Ribadu et al.,1992), although this agreement was not found as reported (Ott et al.,1977, Watson & Munro,1989). Others did not find that corpus luteum quality exerts an influence on the results of the transfers (Donaldson,1985) and have shown that transfer one may perform by palpable corpus luteum of any quality formed following observed estrus (Nelson & Nelson,1985). Several studies have indicated the dependence of the results of the transfers on circulated progesterone levels (Northey et al.,1985), whereas a close correlation between serum progesterone levels on the day of embryo transfer and pregnancy the rates of recipients was not observed (Hahn et al.,1982, Nelson & Nelson,1985).

Rectal palpation is a widely used technique for diagnosing ovarian status in cattle. The criteria for the diagnosis of the functional activity of the corpus luteum by palpation include its diameter and firmness, a demarcation line between luteal tissue and ovarian stroma, overall shape, changes in luteal tissue firmness and ovulatory crown as morphological evidence of ovulation (Belling,1986, Coleman et al.,1987, Pieterse et al.,1990), although over 50% of corpora lutea do not have a palpable crown (Coleman et al.,1987).

It has been reported that palpation may not be a good diagnostic method for the prediction of corpora lutea activity (Ott et al.,1977), while others report the detection of mid-cycle corpora lutea by palpation to predict high progesterone level was good and identical to results obtained by ultrasonography (Spreher et al.,1989, Pieterse et al.,1990).

Our investigation was conducted to evaluate by rectal palpation the functional activity of corpora lutea formed in synchronized recipients on the day of embryo transfer and compare the accuracy with blood serum radioimmunoassay.

Materials and methods

1486 virgin heifers of Estonian Red and Estonian Black and White breeds (15—16 mo of age, 310—350 kg bodyweight) were used as the potential recipients. The estrus of the recipients was synchronized using two injections of cloprostenol (Oestrofan, SPOFA) at an interval of 11 days. Days of estrous cycles of the

heifers at the first cloprostenol injection were unknown and no signs of estrus were observed on the heifers following the second administration of cloprostenol. The ovaries of all the potential recipients were rectally palpated on the day of embryo transfer (D 7 after insemination of donors) to determine the common ovarian status, the presence of corpora lutea, and to evaluate their quality and functional activity.

Depending on morphological characteristics the corpora lutea were classified in quality as very good (VG) and good (G) — high progesterone levels and as poor (P) — low progesterone level. All palpations of recipients were performed through study by one specialist experienced in the technique of palpation. As a standard, the blood serum progesterone radioimmunoassay (RIA) was used to evaluate the accuracy of the diagnosis.

Blood samples (10 ml) were collected from 130 recipients by jugular venipuncture, allowed to clot at room temperature and then stored in a refrigerator overnight before being assayed. A serum progesterone RIA kit STERON-P (IBC, Belorus) was used according to the manufacturer's descriptions. The radioactivity was counted by Gamma-Track 1290 (Tracor Analytic, Elc Grove Village, IL) and progesterone concentrations were calculated using logit-log transformations. A progesterone value in blood serum 2.0 ng/ml on the day of embryo transfer was considered as the norm criterion (Hahn et al.,1982, Northey et al.,1985). Relative to the norm criterion, the progesterone levels of recipients were recorded as high if were equal or higher than 2.0 ng/ml and as low if the level of the hormone was less than 2.0 ng/ml.

The transfer of embryos to 434 recipients were performed non-surgically to the uterine horn ipsilateral to the current corpus luteum within 3—5 h after embryo recovery and storage at room temperature in Dulbecco's medium with 20% fetal calf serum. All transferred embryos were classified as being in the morula and blastocyst stage of development and estimated as good and excellent in quality. The pregnancy of the recipients was determined by routine palpation of the uterus per rectum 45—60 days following transfers.

Data analysis

Values of progesterone concentrations expressed as the mean ± the standard error of the mean (SEM) were established to confirm their common high or low luteal status. For evaluation of obtained data, Student's t-test was used to compare the means between groups. Data were considered to be significantly different at $P<0.05$.

To evaluate the accuracy of palpation for diagnosing high or low functional activity of corpora lutea formed in recipients on the day of embryo transfer, the predictive value as the probability that the diagnosis is correct, the sensitivity as the ability of the palpation to detect corpus luteum high activity and the specificity as the ability of the palpation to detect corpus luteum low activity were calculated (Sprecher et al.,1989, Pieterse et al.,1990). For calculation the numbers of correct positive diagnosis, incorrect positive diagnosis, correct negative diagnosis and incorrect negative diagnosis were used.

Results

The findings of rectal palpation (Table 1) indicate ovarian status in recipients on the day of embryo transfer. The majority of recipients (91.3%) had corpora lutea different in quality. In 8.7% of recipients corpus luteum had either not formed or ovulation not occured and ovaries had become cystic.

Table 1. *Ovarian status of synchronized recipients on the day of embryo transfer*

Ovarian status of recipients	Number of recipients	%
Cystic ovaries	64	4.3
Absence of corpus luteum	65	4.4
Corpus luteum of poor quality	225	15.1
Corpus luteum of good quality	547	36.8
Corpus luteum of very good quality	585	39.4
Total	1486	100.0

There were considerable differences in blood serum progesterone concentrations among recipients (Table 2). In 93.1% of recipients progesterone fluctuated from 0.21 to 10.16 ng/ml and in 6.9% no luteal activity was found.

Table 2. *Progesterone concentrations in blood serum of synchronized recipients on the day of embryo transfer determined by RIA.*

Progesterone concentrations (ng/ml)	Number of recipients	%
...0.00	9	6.9
0.21...0.27	16	12.2
1.15...1.96	25	19.1
2.00...2.94	26	19.8
3.00...3.94	17	12.9
4.03...4.94	15	11.5
5.12...5.96	11	8.4
6.00...6.94	4	3.1
7.02...7.43	3	2.9
8.42...8.51	3	2.9
9.48...10.1	2	1.5
Total	130	100.0

As it follows from the results of the hormonal analysis (Table 3) there were no significant differences in average progesterone concentrations between groups of recipients bearing corpora lutea classified as very good and good ($P<0.05$). The progesterone concentration in recipients with corpora lutea of poor quality was on an average lower by 2.1 and 1.7 times respectively ($P<0.001$; $P<0.01$).

No differences in pregnancy rates between groups of recipients (Table 4) with high functional activity of corpus luteum ($P>0.05$) were found.

The pregnancy rate of recipients with corpora lutea of low functional activity was 28.4 and 20.7% less respectively than the pregnancy of recipients having corpora lutea classified in quality as very good and good ($P<0.001$ and $P<0.05$).

Progesterone concentrations <2.0 ng/ml had been found in 21.7 and 22.7% of recipients with very good and good corpora lutea and the high progesterone

levels (≥2.0 ng/ml) had been found in 35.5% of recipients with corpora lutea of poor quality (Table 5).

Table 3. *Average blood serum progesterone concentrations in the groups of recipients having corpora lutea different in quality.*

Corpora lutea quality	Number of recipients (n=121)	Progesterone concentrations (ng/ml)	Range
VG	46	3.95±0.35	0.22...10.1[a]
G	44	3.25±0.25	0.21...6.96[b]
P	31	1.86±0.21	0.27...3.27[c]

[ab] *Difference not significant (P>0.05)*
[ac, bc] *Differences highly significant (P<0.001; P<0.01)*

Table 4. *Pregnancy rates of recipients in the presence of the corpora lutea different in quality.*

Corpora lutea quality	Number of recipients	Pregnancy rate	
		No	%
VG	308	209	67.9[a]
G	83	50	60.2[b]
P	43	17	39.5[c]

[ab] *Difference not significant (P>0.05)*
[ac, bc] *Differences highly significant (P<0.001; P<0.01)*

Table 5. *Functional activity of corpora lutea different in quality on the day of embryo transfer.*

Corpora lutea quality	Number of recipients	Progesterone concentrations			
		high ≥2.0 ng/ml		low <2.0 ng/ml	
		No	%	No	%
VG	46	36	78.3	10	21.7
G	44	33	77.8	10	22.7
P	31	11	35.5	20	64.5

The prediction accuracy of the high and the low functional activity of the corpora lutea in recipients by rectal palpation on the day of embryo transfer relatively to the results of blood serum RIA is given in Table 6 and Table 7.

Table 6. *The prediction accuracy of the high luteal activity by rectal palpation of corpora lutea relatively to the results of blood serum RIA.*

Palpation diagnosis	Progesterone levels by RIA		Total
	high	low	
High progesterone	70[a]	20[b]	90
Low progesterone	11[c]	20[d]	31
Total	81	40	121

Positive Predictive Value [a/(a+b)]100=77.8%
Sensitivity [a/(a+c)]100=86.4%
Specificity [a/(b+d)]100=50.0%

Using in our study a value of 2.0 ng progesterone/ml in blood serum as the norm criterion for embryo transfer, there were significant differences in pregnancy rates between recipients (Table 8). Recipients with progesterone levels less than 2.0 ng/ml had a 25.5% lower conception rate than recipients which had progesterone levels ≥2.0 ng/ml (P<0.05).

Table 7. *The prediction accuracy of the low luteal activity by rectal palpation of corpora lutea relatively to the results of blood serum RIA.*

Palpation diagnosis	Progesterone levels by RIA		Total
	high	low	
High progesterone	20[a]	11[b]	31
Low progesterone	20[c]	70[d]	90
Total	401	81	121

Positive Predictive Value [a/(a+b)]100=64.5%
Sensitivity [a/(a+c)]100=50.0%
Specificity [a/(b+d)]100=86.4%

Table 8. *Dependence of pregnancy rates of recipients on progesterone concentrations in blood serum on the day of embryo transfer.*

Progesterone concentrations	Number of recipients	Pregnant recipients	
		No	%
<2.0 ng/ml			
0.21...1.30	12	4	33.3
1.31...1.94	20	9	45.0
Total	32	13	40.6[a]
≥2.0 ng/ml			
2.00...2.94	25	16	64.0
3.10...3.94	16	10	62.5
4.03...4.94	15	11	73.3
5.12...5.96	12	9	75.0
6.30...10.1	11	6	54.5
Total	79	52	65.8[b]

[ab] *Difference significant (P<0.05)*

Discussion

In the present study an attempt has been made to identify heifers synchronized by two cloprostenol injections as suitable and unsuitable for the transfer of embryos by rectal evaluation of the activity of the corpora lutea (high or low) on the day of embryo transfer. The day of estrus in recipients after the second cloprostenol injection was unknown.

On the day of embryo transfer ovarian status was different among animals and corpora lutea of different quality were formed in 91.3% of recipients. The functional activity of corpora lutea classified by palpation as very good and good were determined by RIA as similar and commonly it confirmed their high functional activity as predicted. The corpora lutea in 15.1% of recipients were evaluated by palpation as poor in quality (low functional activity) and considered on average as inadequate for embryo transfer. However, as defined by hormonal analysis progesterone levels were less than 2.0 ng/ml in 21.7 and 22.7% of recipients having corpora lutea classified as very good and good, whereas in 35.5% of recipients with poor corpora lutea progesterone concentrations were 2.0 ng/ml and more.

Since, in determination of the high luteal activity, the predictive value was 77.8% that is rather lower than

the accuracy reported (Spreher et al.,1989) and similar to results obtained in evaluation of the mid-cycle corpus luteum (Pieterse et al.,1990). More misdiagnoses in our study have been made to predict by palpation the low luteal activity. Incorrect predictions have been made in the presence of indistinct morphology in ovaries (the poor corpus luteum) which, however, in 35.5% produced progesterone more than 2.0 ng/ml. The predictive value 64.5% is similar to the accuracy in the prior reports (Spreher et al.,1989). The sensitivity in our study is, however, lower.

The pregnancy rates of recipients related in our study to the concentrations of progesterone in periferal blood on the day of embryo transfer. Our data on the use of progesterone levels 2.0 ng/ml as the norm criterion for the transfer of embryos is in agreement with the findings reported (Northey et al.,1985) and can be useful for differentiating between suitable and unsuitable recipients.

We can conclude that the identification of heifers as good or bad recipients based on a determination of corpus luteum quality related to the results of blood serum RIA in 77.8% of recipients evaluated as proper and 64.5% as bad. The highest pregnancy rates were obtained after the transfer of embryos to recipients having corpora lutea classified in quality as good and very good. Then, the selection of potential recipients based on the determination of corpora lutea quality presented the possibility of having approximately 80% of animals proper for embryo transfer.

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Helminths Of The Most Important Game In Estonia

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Abstract

*As a result of an investigation into the game helminthofauna 40 species of helminths in artiodactyls were diagnosed. Roe deer (*Capreolus capreolus*) were invaded with 28, moose (*Aces alces*) with 11, red deer (*Cervulus elaphus*) with 4 and wild boar (*Sus scrofa*) with 9 species of helminths. Chabertiosis, ostertagiosis and nematodirosis were the most frequent helminthoses in roe deer. The most requent helminths found in moose were* Nematodirella alcidis *and* Taenia hydatigena, larvae. *The most frequent helminthosis in wild boar was metastrongylosis.* Trichinella nativa *and* T. britovi *were identified in wild boar.*

Key words: Roe deer, moose, wild boar, red deer, helminthofauna

The most important game in Estonia are moose (*Alces alces*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). Helminthoses are one of the reasons for a decline in game production and its quality. One should take several common species of helminths in *Cervidae* and domestic ruminants into consideration. We can assume the transmission of helminths in particular from roe deer to sheep. Some game helminthoses, especially trichinellosis, are a danger to human health.

This study was conducted in 1973—1993. Altogether 214 animals from different parts of Estonia were examined for helminths, of these 142 roe deer, 37 wild boar, 30 moose and 5 red deer

(Fig. 1). Of them 181 animals were shot and 33 were found dead in the forest.

Systematic part

Class: *TREMATODA*

1. *Parafasciolopsis fasciolaemorpha* Ejsmont, 1932

Found in liver and the small intestine of roe deer.

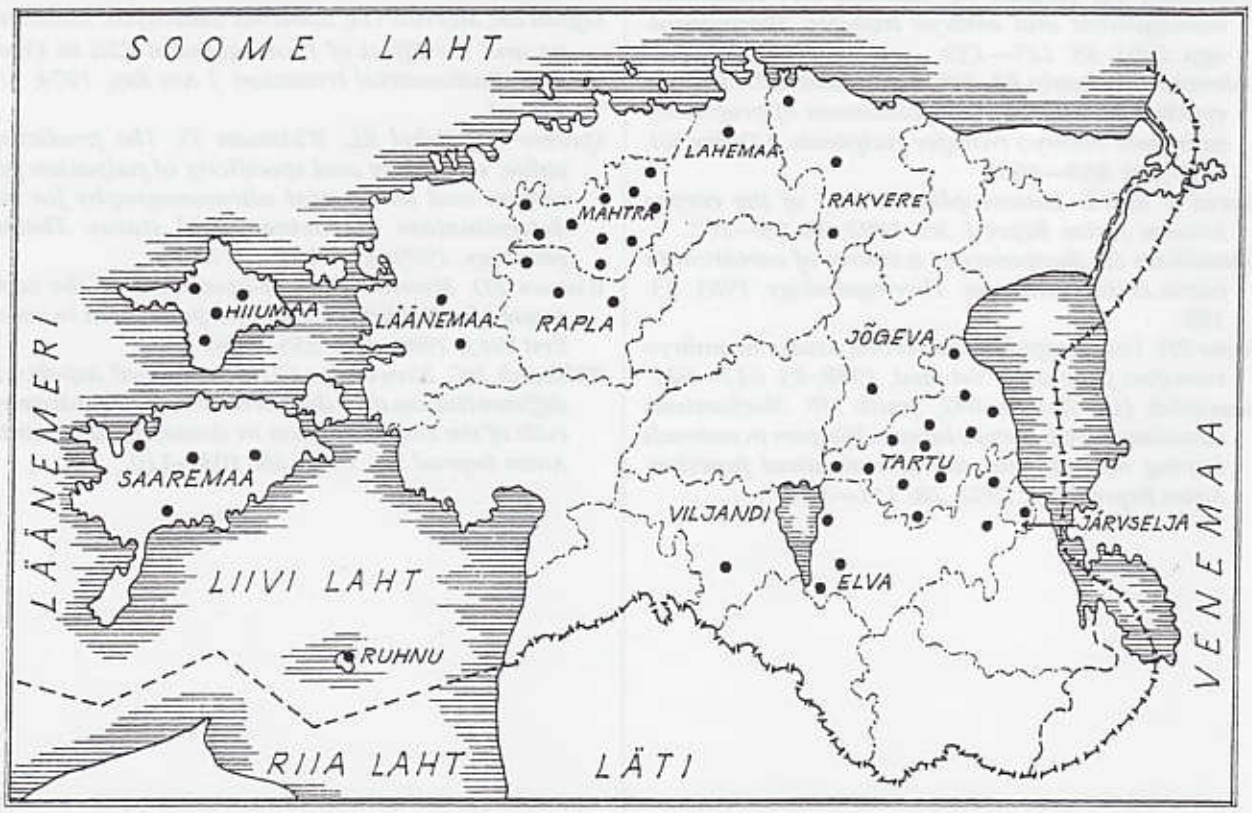

Figure 1. *The places of investigations.*

2. *Dicrocoelium dendriticum* (Rudolphi, 1819)
Found in liver and in the small intestine of roe deer.

Class: *CESTODA*
1. *Moniezia expansa* (Rudolphi, 1810)
Found in the small intestine of roe deer.
2. *M. benedeni* (Moniez, 1879)
Found in the small intestine of moose.
3. *M. sp.*
Found in the small intestine of roe deer.
4. *Taenia cervi, larvae* Christiansen, 1931
Found under the epicardium of roe deer.
5. *Taenia hydatigena, larvae* (Pallas, 1766)
Found on the omentum and liver of roe deer, moose, red deer and wild boar.

Class: *NEMATODA*
1. *Trichocephalus ovis* Abildgaard, 1795
Found in the caecum of roe deer.
2. *T. skrjabini* (Baskakow, 1924)
Found in the caecum of roe deer.
3. *T. globulosa* Linstow, 1901
Found in the caecum of roe deer and moose.
4. *T. suis* Schrank, 1788
Found in the caecum of wild boar.
5. *Aonchotheca bovis* (Schnyder, 1906)
Found in the small intestine of roe deer.
6. *Trichinella sp., larvae* Railliet, 1895
Found in striated suscies of wild boar.
7. *Bunostomum trigonocephalus* (Rudolphi, 1808)
Found in the small intestine of roe deer, moose and red deer.
8. *Chabertia ovina* (Fabricius, 1788)
Found in the colon and caecum of roe deer.
9. *Oesophagostomum dentatum* (Rudolphi, 1803)
Found in the colon of wild boar.
10. *Oe. radiatum* (Rudolphi, 1803)
Found in the colon of roe deer.
11. *Oe. venulosum* (Rudolphi, 1809)
Found in the colon of roe deer and red deer.
12. *Dictyocaulus viviparus* (Bloch, 1782)
Found in the bronchi and trachea of roe deer.
13. *Metastronylus elongatus* (Dujardin, 1845)
Found in the bronchi of wild boar.
14. *M. pudendotectus* Wostokow, 1905
Found in the bronchi of wild boar.
15. *M. salmi* Gedoelst, 1923
Found in the bronchi of wild boar.
16. *Protostrongylus sp.* Kamensky, 1905
Found in the bronchi of roe deer.
17. *Protostrongylus capreoli, larvae* (Stroh et Schmid, 1938)
Found in the parasitic noduli of roe deer and moose.
18. *Trichostrongylis capricola* Ransom, 1907)
Found in the abomasum and small intestine of roe deer.
19. *T. colubriformis* Giles, 1892
Found in the abomasum and small intestine of roe deer and moose.
20. *Haemonchus contortus* (Rudolphi, 1803)
Found in the abomasum of roe deer.
21. *Nematodirell longissimespiculata* (Romanovitsch, 1915)
Found in the small intestine of moose.
22. *N. alcidis* (Dikmans, 1935)
Found in the small intestine of moose.
23. *Nematodirus filicollis* (rudolphi, 1802)
Found in the small intestine and abomasum of roe deer.
24. *N. erschowi* Nasarova et Järvis, 1980
Found in the small intestine of roe deer and red deer.
25. *Ostertagia leptospicularis* Assadov, 1953
Found in the abomasum of roe deer and red deer.
26. *O. antipini* Matschulsky, 1950
Found in the abomasum of moose.
27. *Rinadia mathevossiani* (Ruchljadev, 1948)
Found in the abomasum of roe deer.
28. *Skrjabinagia kolchida* (Popova, 1937)
Found in the abomasum of roe deer.
29. *Spiculopteragia spiculoptera* (Guschanskaja, 1931)
Found in the abomasum of roe deer.
30. *S. dagestanica* (Altajev, 1953)
Found in the abomasum of roe deer and moose.
31. *Ascaris suum* Goeze, 1782
Found in the small intestine of wild boar.
32. *Physocephalus sexalatus* (Molin, 1860)
Found in the stomach of wild boar.
33. *Setaria transcaucasica* Assadov, 1952
Found in the abdominal and thoracic cavity of roe deer and moose.

In total 40 species of helminths were found in the animals examined: 18 in roe deer, 11 in moose, 4 in red deer and 9 in wild boar (Järvis, 1993). We could not identify one of the cestodes (neither *Moniezia expansa* nor *M. benedeni*) and we labelled it as *Moniezia sp.* The muscle samples taken from wild boars were sent to the international Trichinella Reference Centre were *Trichinella nativa* and *T. britovi* were identified (Pozio, Bandi, La Rosa, Järvis, Miller, Kapel, 1995). We diagnosed larvae taken from the majority of samples as *Trichinella sp. Nematodirus erschovi* Nazarova et Järvis nov, sp. was described as a new helminth species in roe deer (Nazarova, Järvis, Puzauskas, 1980).

A review of the helminthofauna

1. It was established that 96% of the roe deer examined had been invaded with helminths. The number of helminth species in the invaded roe deer was 1—12 (on average 5). The helminth species occuring the most frequently in roe deer were as follows: *Ostertagia leptospicularis, Chabertia ovina, Bunostomum trigonocephalum* and *Nematodirus filicollis* (Table 1). Taking the intensity of helminth invasion, pathological changes and clinical symptoms (diarrhoes, emaciation) in roe deer into consideration we can conclude that the most important helminthoses in roe deer in Estonia are chabertiosis, ostertagiosis and nematodirosis. Careful examination of the roe deer corpses revealed that 18% of the deaths were caused by the intensive invasion of helminths.

2. All the moose examined were invaded with helminths (Järvis, 1995). The number of helminths species in the invaded moose was 1—8 (on average 5). The helminths found quite often in the moose were as follows: *Nematodirella alcidis, Taenia hydatigena,*

larvae and *Ostertagia antipini* (Table 2). *Cysticercus tenuicollis* has frequently been found on the omentum (2—7 cysts, from 1,5 cm in diametre to the size of goose's egg). Six cestodes *Moniezia benedeni* (length up to 66 cm) were found in the small intestine of one moose. Parasitic noduli (up to 2,5 cm in diametre) containing larvae and eggs of *Varestrongylus capreoli* in large numbers were found in pulmonary tissue and under the pleura.

Table 1. *Helminth invasion in roe deer.*

Helminths	Prevalence %	Intensity of invasion		
		min	max	mean
Parafasciolopsis fasciolaemorpha	1	17	17	17
Dicrocoelium dendriticum	16	3	186	34
Moniezia expansa	4	1	3	1
M. sp.	3	1	2	2
Taenia cervi, larvae	1	1	1	1
Taenia hydatigena, larvae	7	1	20	4
Trichocephalus ovis	11	1	8	3
T. skrjabini	1	1	1	1
T. globulosa	37	1	42	6
Aonchotheca bovis	1	2	2	2
Bunostomum trigonocephalum	53	1	83	16
Chabertia ovina	65	1	615	119
Oesophagostomum radiatum	1	1	3	2
Oe. venulosum	3	1	1	1
Dictyocaulus viviparus	28	1	170	23
Protostrongylus sp.	1	2	2	2
		parasitic noduli:		
Varestrongylus capreoli	12	2	8	4
Trichostrongylus capricola	32	2	125	21
T. colubriformis	3	9	80	34
Haemonchus contortus	4	1	9	4
Nematodirus filicollis	46	1	1600	146
N. erschowi	6	2	400	69
Ostertagia leptospicularis	85	3	1200	136
Rinadia mathevossiani	1	1	1	1
Skrjabinagia kolchida	1	1	6	24
Spiculopteragia spiculoptera	9	1	23	10
S. dagestanica	6	1	16	6
Setaria transcaucasica	26	1	38	6

3. 60% of the red deer examined were invaded with helminths. The number or helminth species in the invaded red deer was 1—3 (on avarage 2). The invasion with helminths was of low intensity (Table 3).

4. 95% of the wild boars examined were invaded with helminths. The number of helminths species in the invaded wild boar was 1—7 (on average 3). The most frequent helminthosis in wild boars was metastrongylosis (Table 4). Wild boar meat is the main source of human trichinellosis in Estonia. In nature raccoon dogs (*Nyctereutes procyonoides*), foxes (*Vulpes vulpes*), lynxes (*Felis lynx*), wolves (*Canis lupus*), badgers (*Meles meles*) etc. are key figures in connection with this serious disease. Hunters can also help the spread of trichinellosis by leaving the corpses of game in the forest or by feeding such raw meat to dogs, cats and domestic pigs.

Table 2. *Helminth invasion in elk.*

Helminths	Prevalence %	Intensity of invasion		
		min	max	mean
Moniezia benedeni	17	1	6	2
Taenia hydatigena, larvae	70	1	7	3
Trichocephalus globulosa	50	4	21	13
Bunostomum trigonocephalum	33	15	46	28
		parasitic noduli:		
Varestrongylus capreoli	30	2	7	4
Trichostrongylus colubriformis	23	10	14	12
Nematodirella longissimespiculata	43	5	200	52
Nematodirella alcidis	70	7	80	21
Ostertagia antipini	63	8	38	22
Spiculopteragia dagestanica	30	3	5	4
Setaria transcaucasica	20	2	4	3

Table 3. *Helminth invasion in red deer.*

Helminths	Prevalence %	Intensity of invasion		
		min	max	mean
Taenia hydatigena, larvae	20	1	1	1
Bunostomum trigonocephalum	20	2	2	2
Oesophagostomum venulosum	20	2	2	2
Ostertagia leptospicularis	60	3	11	9

Table 4. *Helminth invasion in wild boar.*

Helminths	Prevalence %	Intensity of invasion		
		min	max	mean
Taenia hydatigena, larvae	3	1	1	1
Trichocephalus suis	68	2	25	8
Trichinella sp.	3	8 larvae p.g.		
Oesophagostomum dentatum	8	2	5	3
Metastrongylus elongatus	89	2	85	18
M. pudendotectus	92	3	74	19
M. salmi	30	2	12	7
Ascaris suum	30	1	3	2
Physocephalus sexalatus	14	1	12	4

5. The helminthofauna of wild artiodactyls is formed on the basis of different natural and anthropogenic factors. Of the anthropogenic factors, shooting is of great importance. This enables us to aschieve the optimum population size and remove the diseased and feeble animals from the herd. The frequent preva-

lence of tenuicollis-cysticercosis in game is due to carnivores including dogs infected with cestodes. Helminths can be transferred from wild to domestic ruminants, especially from roe deer to sheep. The extensive spread of strongyloidoses can be explained by that. In Estonia the hazard of metastrongylosis continues to rise in connection with the increasing outdoor keeping of domestic swine.

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Ultrasonic Measurement Of Animal Eye Dimensions

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Abstract

A biometric study, using A-mode ultrasonography, was done on 14 eyes of bulls aged from 16 to 18 month, 12 eyes of pigs aged from 8 to 9 month and 12 eyes of 2 days-old piglets in vitro. Five intraocular dimensions were measured, including the distance from the anterior cornea to the anterior lens surface, the lens thickness, the distance from the posterior lens surface to the retina, distance from the anterior cornea to the retina and corneal thickness. Statistical difference of all intraocular dimensions between the pigs and piglets eye was established. Percentage relation between axis of eye- ball and others intraocular dimensions of the bulls, pigs and piglets was determined.

Key words: A-scan ultrasonography; ocular biometry; eye, bull, pig, piglet.

Introduction

Ocular biometry using A-scan ultrasonography has been performed in several studies in veterinary ophtalmology (Schiffer et al. 1982, Cottrill et al. 1989, Ekesten 1993). Ultrasonography has diagnostic potential in veterinary ophtalmology, but its use has been limited by the paucity of reports of normal ultrasonographic ocular and orbital anatomic features. A-scan ultrasonography has been used for almost 20 years for biometry and as a diagnostic aid, especially when the refractive media (lens, cornea) is opaque (Cottrill et al. 1989). The purpose of performing ocular biometry has mainly been to either determine the axial length for calculation of the power of artificial intraocular lenses or to measure the sagital size of intraocular structures in canine glaucoma. Further, ocular biometry is now in frequent use at certain veterinary clinics (Ekesten 1993). Both A-scan and B-scan ultrasonography has been used for ocular biometry in euthanised dogs and it was found that results were similar (Cottrill et al. 1989). However, it has been stated that A-scan is more accurate than B-scan for measuring intraocular distances (Coleman 1979). The bias of ocular biometry using A-scan has been studied in a number of investigations (Schiffer et al. 1982). Thus, correction for bias caused by different velocities of ultrasound in different parts of the eye can be performed, since the true distances have been determined with other methods, and constants of conversion are known (Jansson 1963, Schiffer et al. 1982).

Various aspects of aging of the eye have been investigated in different mammal species. Age-related histologic and biochemical changes, as well as physiologic alterations, in intraocular structures have been described. The sagital growth of the normal eye and intraocular structures has been studied in human beings, rhesus monkeys, and tree shrews. The sagital growth of different parts of the eye is of interest for understanding the development of refractive errors and age-related diseases, such as primary angle-closure glaucoma (Ekesten 1995). Studies are done on human changes, such as a reduction of total axial length, increased lens thickness, and reduction of the anterior chamber. The statistical analysis revealed no differences between the axial diameters for the right and left eye of the dogs. However, a statistical comparison of the male and female total axial length did show a significant increase of the male over the female eye. The body weights of the dogs were closely matched, with the average male body weight being 20,73 kg and the average female body weight being 20,46 kg (Schiffer 1982).

The purpose of the present report was to establish an efficient technique for applying ultrasound to the animal eye and to establish reference values for the animal eye for such distances as the cornea to lens, lens thickness, lens to retina, and total axial length.

Material and methods

We have realized this work in cooperation with the Laboratory of Ophtalmology of Kaunas Medical Academy and Kaunas University of Technology. Using A-mode ultrasonography, we investigated structural parameters of the eye, depending on the species and the age of the animals. The intraocular dimensions measured were as follows: distance between the anterior cornea to the surface of the anterior lens, thickness of the lens, distance between the surface of the posterior lens and surface of the retina, distance from the anterior cornea to the retina, which represents the total axial and central corneal thickness.

We have designed the original investigation methods and ultrasonic equipment with ultrasonic transducer (f=12 Mhz). Using the acoustodiagnostic system, we were carrying out precise biometry of eye tissues.

Ultrasonic investigations were done on 14 eyes of bulls aged from 16 to 18 month, 12 eyes of pigs aged from 8 to 9 month and 12 eyes of 2 days-old piglets *in vitro*.

Results

Data about measurements of the ocular dimensions are presented in Tables 1—3. There was a significant increase of the ocular dimensions of the eye in the bulls over that of the eye in the pigs.

Table 1. *Ocular measurements (mm) of the bulls.*

Case	Axial length	Corneal thickness	Depth of the anterior chamber	Thickness of the lens	Axial length of the vitreous
1	38.4	1.3	6.2	12.6	20.4
2	37.3	1.3	6.4	6.3	24.9
3	37.0	1.2	6.2	7.9	22.4
4	36.8	1.2	6.2	12.1	22.2
5	34.2	1.3	5.8	12.3	16.4
6	34.3	1.3	5.9	12.4	16.5
7	34.5	1.3	6.0	10.5	16.5
8	32.5	1.2	5.9	10.9	15.9
9	32.5	1.4	5.6	10.7	16.3
10	30.8	1.4	5.4	11.2	14.6
11	31.7	1.2	5.7	7.9	15.2
12	32.5	1.3	3.4	7.4	15.7
13	32.4	1.3	3.3	7.7	15.4
14	32.1	1.2	3.4	7.9	15.1
n=14	34.07±0.64	1.28±0.02	5.38±0.3	9.84±0.59	17.68±0.89

Table 2. *Ocular measurements (mm) of the pigs.*

Case	Axial length	Corneal thickness	Depth of the anterior chamber	Thickness of the lens	Axial length of the vitreous
1	23.0	1.1	3.6	6.2	13.5
2	27.2	1.1	3.9	7.7	15.2
3	24.6	1.1	3.6	7.1	14.1
4	25.0	1.1	3.7	7.7	13.8
5	25.3	1.1	3.7	7.7	13.5
6	25.2	1.1	3.7	7.7	13.4
7	25.4	1.1	3.8	7.8	13.6
8	25.3	1.1	3.8	7.7	13.5
9	24.7	1.1	3.6	7.0	9.6
10	22.7	1.1	3.7	7.3	11.9
11	21.9	1.1	3.6	7.3	11.0
12	21.7	1.2	3.6	7.3	10.8
n=12	24.33±0.48	1.10±0.008	3.7±0.03	7.37±0.13	12.82±0.47

A comparison of the mean ocular measurements of the pigs' eyes compared with the mean ocular measurements of the piglets is presented in Table 4. A statistical difference between pigs' and piglets' ocular dimensions ($p<0,001$) was found. Only statistical difference was not found was that of the corneal thickness ($p>0,05$).

The percentage relation between total axial length and other ocular dimensions is presented in Table 5. There were no significant differences of percentage relation between bulls' and pigs' ocular dimensions. But we found a significant difference of percentage relation depending on the age. The percentage relation between axial length and corneal and lens thickness of piglets show that the cornea and lens occupy much more of the total axial length than in the bulls' and pigs' eyes.

Table 3. *Ocular measurements (mm) of the piglets.*

Case	Axial length	Corneal thickness	Depth of the anterior chamber	Thickness of the lens	Axial length of the vitreous
1	13.9	1.3	1.1	4.8	5.3
2	13.4	1.3	1.5	5.3	5.3
3	14.1	1.2	1.4	4.8	7.2
4	13.4	1.1	1.7	4.9	6.2
5	13.1	1.4	1.9	5.2	5.8
6	13.8	1.1	2.0	5.4	6.3
7	13.2	1.1	1.9	4.9	5.8
8	13.1	1.3	1.7	5.1	5.9
9	13.6	1.2	1.7	5.1	6.1
10	13.5	1.2	1.9	5.3	6.2
11	13.8	1.0	1.5	4.9	7.1
12	14.0	0.9	—	4.9	7.2
n=12	13.56±0.09	1.17±0.04	1.68±0.08	5.05±0.06	6.2±0.19

Table 4. *Comparison of the ocular measurements (mm) of pigs and piglets.*

	Axial length	Corneal thickness	Depth of the anterior chamber	Thickness of the lens	Axial length of the vitreous
Pigs (n=12)	24.33±0.48	1.10±0.008	3.7±0.03	7.37±0.13	12.32±0.47
Piglets (n=12)	13.56±0.09	1.17±0.04	1.68±0.08	5.05±0.06	6.2±0.19
p	<0.001	>0.05	<0.001	<0.001	<0.001

Table 5. *Percentage relation between total axial length and other ocular dimensions.*

	Cornea	Anterior chamber	Lens	Vitreous
Bulls	3.76	15.701	28.882	51.893
Piglets	8.63	12.389	37.241	45.722
Pigs	4.52	15.208	30.291	52.692

Discussion

The literature on the morphological features of the eye depending on age in different domestic animals is meagre. Some authors (Gellat 1981, Slatter 1990) have described ocular parameters without indicating changes according to age.

The purpose of the present work is to establish a technique for applying ultrasound methods to the animal eye, and to establish reference values for the animal eye for such distances as the anterior cornea to lens, lens thickness, lens to retina, total axial length and corneal thickness, depending on the species and age of the animals.

The present method, using a saline bath supported by a plastic membrane, was chosen because of its easy application and neatness. However, the saline bath was lowered only far enough to achive good contact with the cornea. Also, the presence of the plastic membrane could have affected the initial echo at the corneal surface. The transducer used in the present

study was selected for its relatively high frequency (f=12 Mhz) and wide focusing range, along with its ability to perform A-mode ultrasonography. This transducer's versality was desired in anticipation of possible studies on the eye in various species. The wave nature of ultrasound is similar to that of light, ultrasonic waves follow similar principles of behaviour, including reflection, refraction, and absorption. In addition, ultrasound can be focused. As the waves travel, they partially reflect at interfaces between tissues of differing acoustic impendence. The major interfaces in the eye are at the cornea, the anterior lens surface, the posterior lens surface and the retina. The aqueous lens substance and vitreous are relatively homogeneous materials and therefore do not interrupt the propagation of ultrasonic waves.

The ocular dimensions determined in the present study, such as the lens thickness, were compared among bulls, pigs and piglets. There was a significant increase in the ocular dimensions of the eye in the bulls over that of the eye in the pigs. A statistical difference between pigs' and piglets' ocular dimensions ($p<0,001$) was found. The only statistical difference not found was that of the pigs' and piglets' corneal thickness ($p<0,05$). There were no significiant differences of percentage relation between bulls' and pigs' ocular dimensions. But, however, we found a significant difference in the percentage relation depending on the age. The percentage relation between axial length and corneal thickness of piglets shows that the cornea occupy much more of the total axial length than in the bulls' and pigs' eyes (Fig. 1).

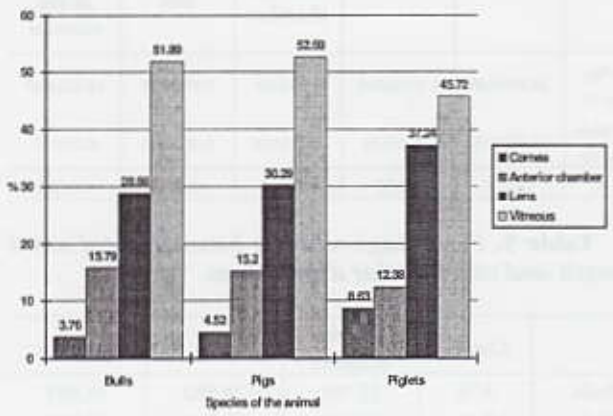

Figure 2. *Percentage relation between axial length and other ocular dimensions of bulls, pigs and piglets.*

An important function of the present study was to initiate interest in biometric and diagnostic ocular ultrasonography and to get information about the eye-ball structural parameters and changes of them in relation to age. In the past, clinical application of A-mode ultrasonography to the eye in veterinary medicine has been limited. Some authors (Koch et al. 1969) introduced the possibility of using ultrasonography for diagnostic purposes in ophtalmologic studies.

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Isolation Of Canine Parvovirus In Cell Culture And Investigation Of Its Properties During Experimental Infection

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Abstract

The canine parvovirus CPV-2 was isolated in the primary cell culture of a feline kidney from samples of faeces. For identification of the virus, the hemagglutination (HA) and hemagglutination inhibition (HI), and methods of electron and immunoelectron microscopy, were used. The isolate of virus of the fourth passage reached the HA titre 11 log_2. It was used for infection of puppies aged 2—4 months. The virus was detected in faeces on the 4th—5th day after infection and this process lasted for 6 days. The highest HA titres of the virus reached 6,6—8,2 log_2. First titres of antibodies were detected by HI on the 7 th day after infection and they exceeded the titre of collostral antibodies four times. The group of mature dogs responded to the infection rather weakly and excreted no parvoviruses with faeces, whereas the increase of HI titres was negligible. Seroconversion was only established in two dogs in the aforesaid group.

Key words: parvovirus, canine, isolation, experimental infection.

Introduction

The first reports of the canine parvovirus infectious agent (CPV) were registered in 1978 in the USA, Australia, Canada and, shortly after, in Europe [11,12]. This virus is now known as CPV-2. It was successfully isolated from the feces of dogs having hemorrhagic enteritis [1,3,6]. However, since 1967 parvovirus CPV-1 isolated from dog intestines, otherwise called minute virus of canines, has been known since 1967 and it differs from the previously mentioned one in its low pathogenic activity in dogs [11]. At present a certain evolution of CPV-2 can be observed. It is a common opinion that the virus CPV-2 , which earlier had circulated in the USA, Japan and Europe, was replaced in 1980 by a genetic and antigenic variant CPV-2a and CPV-2b. They differ from their predecessor in nucleotides of the 6th gene and amino acids in the protein of capside [9,14].

Parvovirus can be isolated in cell lines: A-72, MDCK, CCL, DKC [1,2,6,8,9,12,14]. There are reports about successful CPV-2 isolation in the cell culture of SPF dog kidney cortex layer [3]. In cell cultures the CPV-2 cytopathic effect is usually unstable. Therefore, supplementary methods of investigation must be used [10].

For identification of the virus isolates hemagglutination (HA) and hemagglutination inhibition (HI) reactions are suggested [1,4,15], using 1% suspension of porcine erythrocytes. Data exist that the specificity of these reactions is higher than that of ELISA and immunofluoressence [8,16]. Electron microscopy (EM) may be applied as a supplementary method of investigation.

The CPV-2 had not been isolated in Lithuania before the present study and canine parvovirus infection was diagnosed only by clinical symptoms and examination of gross lesions. Therefore, the aim of this work was to isolate CPV-2 from the faeces of infected dogs and to examine their properties during experimental infection using dogs of different age as a biological model. Data on experimental infection of dogs is very scarce in literature. The greatest contribution into this field has so far been made by Carmichael et al. [2,3] who studied the attenuated isolates of parvovirus.

Materials and methods

For the isolation of canine parvovirus we used a primary culture of feline kidney cells which was prepared from trypsinized kidneys of kittens aged 2 weeks—1 month in 199 medium containing 5—10% fetal bovine blood serum. The cultures were infected in the beginning and final stage of cell monolayer formation. Infected cells were incubated for 7—8 days at 37 °C and changes in culture throughout the mentioned period were evaluated. For parvovirus isolation, 10 samples of dog feces were used in which parvovirus were detected by hemagglutination (HA) and hemagglutination inhibition (HI). The samples

were selected in which HA titre was not less than 1:512. They were suspended in phosphate buffered saline solution (PBS, pH=6,5) and centrifuged at 500 g for 15 min. The suspension was then treated with chloroform for 10 min and again for 15 min centrifuged at 500 g. To the obtained suspension 100 IU/ ml of penicillin and 100 µg/ml streptomycin were added and then after the cell culture was infected.

Fecal samples and every passage of isolates were investigated by HA and HI reactions, according to Carmichael [4] methods, using 1% suspension of pig erythrocytes.Hyperimmune feline serum to reference mink parvovirus Rodniki strain was used for HI [15]. The parvovirus isolates of the fourth passage were examined by electron microscopy (EM) by the method of negative contrasting [7]. For this purpose the investigated isolates were processed with chlorophorm for 10 minutes and then centrifuged for 30 min at 500 g. The surface layer was concentrated in the centrifuge for 30 min at 35000 g. The precipitate obtained was resuspended with 1—2 drops of distilled water and removed on a copper grid covered with colloid film. The contrasting was accomplished with 2% phosphotungstic acid and after drying the samples were examined by electron microscope EM 125 with magnification 50,000—100,000 times.

For identification of the isolates of fourth passage, immunoelectron microscopy (IEM) with homologous covalescent canine immune serum was performed [16]. For microscopic analysis, 0,8 ml of chloroformed investigated material and 0,2 ml of serum, diluted 1:10 and 1:100, were taken. After incubation and precipitation of the immune complexes, an analysis was done as mentioned above.

For experimental infection, German Sheep-dogs and mixed breeds aged 2—4 months and 2—4 years were used, five in each group. Experimental animals were inoculated per os with 5 ml parvovirus isolate of the fourth passage HA titre which was 1:2048. During the 12 subsequent days the feces of the dogs in each group were examined by HA and the state of health of dogs observed. Blood sera were examined by HI at intervals of 5 days. Dog blood sera were prepared for HI by the method of elimination of thermostable inhibitors with 25% kaolin suspension. Thermolabile inhibitors were inactivated in a 56 °C water bath for 30 min and sera were additionaly processed with 50% pig erythrocytes suspension [10].

Results

Canine parvovirus was isolated only in three samples of faeces with the HA titre being 1:512—1:4096. It was determined that the optimal time for inoculation of kitten kidney cell culture is 8—12 hours after trypsinisation, i.e. before the cell monolayer has completely developed. The average value of HA titres of parvovirus isolates cultivated on such cells was higher than that of isolates obtained from fully developed cell monolayer by 2,7 $\log_2$. Although, cells were incubated for 7—8 days a cytopathic effect of the virus was not observed. In the first passage the HA titre of parvovirus isolates decreased by 1—4 $\log_2$, whereas, in the second through fifth passage it increased by 1—3 $\log_2$ in every passage. In the 5th passage the HA titre of isolates again decreased by 4—5 $\log_2$. The results are given in table 1.

Isolates of the fourth passage were investigated by EM and IEM and identified as parvoviruses. EM revealed that isometric particles of virions were of cubic symmetry but without membranes. Their diameter was 18—20 nm. The diameter of nucleus was 14—17 nm, whereas that of capside was approximately 3—4 nm. These particles are morphologically identical with parvovirus [7]. IEM exposed virions as complexes of a few particles or small aggregates covered with a layer of immunoglobulins.

For experimental infection of dogs we used parvovirus isolate No.1 of the fourth passage with HA titre being 11 $\log_2$. In feces of puppies aged 2—4 months, the virus appeared on the 4th—5th day after infection and was excreted during 6 subsequent days. The highest HA titres of virus in feces were established on the 5th—6th day after challenge and made on average 6,6—8,2 $\log_2$. On the fourth day of the experiment a profuse diarrhoea, vomiting and apathy was observed in two puppies. One of them died on third day. In the remaining three puppies the experimental infection did not cause clinical symptoms and manifested itself only in the virus excreted with feces. The results obtained are given in table 2. In the group of adult dogs feces contained no parvovirus during all 12 days.

Table 1. *HA titres of parvovirus isolates in different passages on cell culture.*

Isolate No	HA titre of feces, $\log_2$	Virus passages and HA titers $\log_2$				
		1	2	3	4	5
1	11.0	10.0	11.0	12.0	11.0	8.0
2	9.0	5.0	8.0	10.0	11.0	7.0
3	12.0	10.0	11.0	10.0	7.0	6.0
M±m	10.6	8.3	10.0	10.6	9.6	7.0
1*	11.0	6.0	7.0	8.0	7.0	6.0
2*	9.0	3.0	6.0	8.0	7.0	7.0
3*	12.0	5.0	7.0	8.0	6.0	6.0
M±m*	10.6	4.6	6.6	8.0	6.6	6.3

*Note: * — passages of virus were accomplished after full development of cell monlayer.*

Table 2. *Parvovirus HA titre in feces of puppies aged 2—4 months.*

Days after infection	Parvovirus HA titres, $\log_2$					
	1*	2*	3	4	5	Mean $\log_2$
1	<1	<1	<1	<1	<1	0
2	<1	<1	<1	<1	<1	0
3	<1	<1	<1	<1	<1	0
4	11	<1	<1	3	<1	2.8
5	12	10	6	5	<1	6.6
6	8	10	10	7	4	8.2
7	9	4	10	5	5	7.8
8	—	3	4	3	6	4.2
9	—	2	3	2	3	2.7
10	—	<1	2	<1	<1	0.5
11	—	<1	<1	<1	<1	0
12	—	<1	<1	<1	<1	0

*Note: * — excretion of virus with feces manifested through diarrhoea.*

In 2—4 month old puppies, high titres of antibodies were established by HI as early as the seventh day after infection. On the 10th day the average value of HI titre was the highest one making 1:24960. Later on it decreased and on the 30th day made 1:1920. It was observed that in blood sera of puppies (No.1 and No.2) who had clinical symptoms of disease, HI titres were higher than in those puppies who had a higher titre of colostral antibodies and who showed no clinical symptoms of infection. The experimental infection revealed that the latter puppies also reacted to infection and antibodies detected in their blood serum on the 7th day after infection by four times as much as the colostral titre of antibodies. The results of the investigations are given in table 3.

Table 3. *Antibody titres in blood sera of puppies aged 2—4 month after experimental infection.*

Days after infection	Puppy number and antibody titre in HI					Mean of antibody titres
	1	2	3	4	5	
0	<1:10	1:20	1:40	1:80	1:160	1:60
7	1:5120	1:20480	1:2560	1:1280	1:640	1:6016
10	—	1:81920	1:5120	1:10240	1:2560	1:24960
15	—	1:10240	1:10240	1:5120	1:2560	1:7040
20	—	1:5120	1:2560	1:1280	1:1280	1:10240
25	—	1:5120	1:1280	1:2560	1:1280	1:2560
30	—	1:5120	1:1280	1:2560	1:1280	1:1920

In the group of dogs aged 2—4 years the increase of HI titres was negligible. Seroconversion was established only in two dogs on the 10th day of the experiment. In remaining dogs antibody titres increased but twice or remained unchanged. By the 20th day following experimental infection HI antibody titres in the blood sera of dogs differed only little from the titres at the beginning of experiment (Table 4).

Table 4. *Antibody titres in blood sera of dogs aged 2—4 years after experimental infection.*

Days after infection	Dog numbers and titres of antibodies in HI			
	1	3	4	5
0	1:640	1:640	1:320	1:640
7	1:640	1:1280	1:640	1:1280
10	1:320	1:640	1:1280	1:2560
15	1:1280	1:640	1:640	1:1280
20	1:640	1:1280	1:640	1:1280

Discussion

Isolates of canine parvovirus in Lithuania do not provoke the cytopathic effect in feline kidneys cell culture. According to Gorski et al [10], Carmichael et al [3] the cytopathic effect provoked by CPV-2 virus is usually unstable. This can be accounted for by virus adaptation in the cell culture, the number of passages, the pathogenecity of the virus. Our investigations reveal that the virus isolates in the primary cultures of feline kidneys propagates well in the first four passages. Virus HA titre in the fifth passage decreases considerably. Therefore, it would be more expedient to adapt parvovirus on the cell lines in which parvovirus is distinguished by its cytopathic effect. According to literary data for CPV-2 virus isolation the cell lines — A 72, MDCK, CCL, KDK — are usable [1,2,3,4,6].

Our investigations revealed that it is important to infect the cell culture before the development of monolayer because in this case HA titres are obtained which are 2,7 $\log_2$ higher than after the infection of cells when the monolayer is completely developed. These results correlate with the data given by Kramer et al [11] which demonstrated that parvovirus replicate well only in those cells which are characterized by active mitosis.

Parvovirus appeared in the feces of experimentally infected puppies aged 2—4 months only on the 4—5th day after infection. Other authors have found the virus in feces already on the 1st—3rd day after infection [1, 6]. However, our observation that parvovirus is excreted with feces for 5—7 days correspond with data given by other authors [1,3,15]. It is also established that clinically healthy dogs may excrete parvovirus with feces. These results correspond with Carmichael et al. [2,3] data according to which in CPV of the fourth passage during experimental infection feces of clinically healthy dogs contained HA titres higher by 6—9 $\log_2$.

The response of adult seropositive dogs to infection was very negligible. Parvovirus excretion in their feces was not observed. This can be accounted for by a high titre of antibodies in their blood sera.

By HI we established that both groups of dogs responded to experimental infection. In puppies aged 2—4 months high titres of antibodies against parvovirus developed on the 7th day of the experiment. It was determined that colostral antibodies may have an effect on the manifestation of disease symptoms and dynamics of HI antibodies titre. When the titre of colostral antibodies is only 1:10-1:20 during experimental infection, clinical symptoms of parvovirus infection occur and after 7 days high titres of antibodies are observed. When the titre of colostral antibodies is 1:40 and higher, the organism response to virus is manifested only through its excretion into the environment and not so distinct seroconversion. Gorski et al [10] established that the titre of colostral antibodies 1:80 and higher interferes occurrence of symptoms of disease. Our results reveal that the value of titre may still be lower.

In the group of infected adult dogs, disease symptoms were not observed. Dogs with high antibody titres (1:320—1:1280) before the experiment responded to the infection weakly. Antibody titres on the 20th day of experiment were the same as before the experimental infection.

Therefore, on the grounds of the results obtained we can assert that canine parvovirus isolated in Lithuania is pathogenic for puppies aged 2—4 months, which have low colostral antibodies level and represents no hazard to seropositive adult dogs.

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An Effect Of Ferolit On The Organism Of Animals

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Abstract

One gram of Ferolit contains 0.0514 g of iron, 0.097 g of copper, 0.581 g of zinc and 0.015 g of cobalt. We have tested various doses of this new composition on the organism of sows, suckling-piglets, rabbits, mice and rats. After seven experiments we determined that Ferolit had a positive influence on haemopoiesis. Analysis of the results of the investigations revealed that the penetration of iron through the digestive tract into the serum of blood increased under the influence of Ferolit. For prophylaxis of anaemia we recommended the use of 13 grams (650 mg Fe) of Ferolit three times for sows or 2 grams (100 mg Fe) for piglets twice on the 3rd and 8th days after birth. This composition was mixed with food on the 10th—14th days before parturition and on the 3rd and 10th—14th days after it. Ferolit can be used only for peroral administration. After treatment with Ferolit, the penetration of iron through the digestive tract into the serum of blood increased by 3.5—4.2% ($P<0.05$), protein by 1.4—1.6% ($P<0.05$), and β-globulin by 14% ($P<0.01$). β-Globulin was incorporated in transferine and it regulated this process. We determined that the level of haemoglobin increased by 1.8—3.3 % ($P<0.05$) and the quantity of erythrocytes by 5,4—12,5 % ($P<0.05$) under the influence of Ferolit. We have not noticed any negative influence of Ferolit on the organism of sows, suckling-piglets, rabbits, mice CBA and Wistar rats. LD_{50} of Ferolit was 1500 mg/kg. Under the influence of Ferolit in doses of 1000 mg/kg and 2500 mg/kg the weight of rats increased by 14—18% ($P<0.01$; $P<0.05$).

Key words: anaemia, suckling-piglets, sows, new composition Ferolit, haematological index, penetration, toxicology, rats, rabbits, mice.

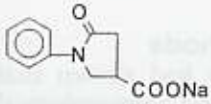
Introduction

Biologically active substances are used in various countries for the regulation of growth, the improvement of the development of an organism, metabolism and haemopoiesis. Anaemia cases are observed in all countries, but causes of this disease are different.

Various compositions of iron and (premix) microelements can be used for prophylaxis and treatment of anaemia. All these compositions can be used perorally, intramuscularly, staining of nipples of milk gland or mixing into the fodder [11, 20]. The damage of anaemia can be 65—70% [4, 15]. Suckling-piglets can get 1/7 part of iron with milk of sows [7]. It is necessary to use iron compositions on the 3rd day after birth [16]. If at the end of pregnancy sows get good quality rations and enough microelements, iron can accumulate in the liver, kidney, spleen, bone marrow and in the placenta [2,19]. For a long time, Lithuania imported iron compositions from abroad, but when the economic conditions changed, this way became very expensive. We decided to find a cheaper solution. We knew what quantity of iron can be in the ration, organism of sows and suckling-piglets, milk, fodder and made a new iron composition in 1991. This composition — Ferolit includes iron, cobalt, copper and zinc.

Characteristics of Ferolit

Ferolit is a mixture of sodium salt of 1-phenyl-4-carboxy-2-pyrrolidinone, ferrous sulphate, zinc sulphate, copper sulphate and cobaltous acetate. Its formula is



, $FeSO_4 \cdot 7H_2O$; $CuSO_4 \cdot 5H_2O$; $(CH_3COO)_2Co \cdot 4H_2O$; $ZnSO_4 \cdot 7H_2O$.

1-Phenyl-4-carboxy-2-pyrrolidinone was obtained by reaction of freshly distilled amine with itaconic acid in boiling water.

In order to eliminate not reacted amine, hydrochloric acid was added to the reaction mixture, the product

was filtered, washed with water and dried. The yield was 85%, melting point 193—194 °C. 1-Phenyl-4-carboxy-2-pyrrolidinone was purified by dissolving it in 5% NaOH solution without heating, filtering and then acidifying the filtrate with hydrochloric acid to pH 4—5. The crystals formed were filtered, washed with water and dried.

Sodium salt of 1-phenyl-4-carboxy-2-pyrrolidinone was obtained by dissolving 1-phenyl-4-carboxy-2-pyrrolidinone in equivalent 17% NaOH solution and filtering if necessary. The density of this solution was 1.205 g/cm^3. It was evaporated to the dry mass. The crystals were dried at 60—100 °C.

The melting point of the salt is 285 °C, formula $C_{11}H_{10}NO_3Na$. The product is very well soluble in water, does not dissolve in acetone, ether, benzene.

Dry sodium salt of 1-phenyl-4-carboxy-2-pyrrolidinone was ground to powder and mixed with ferrous sulphate, zinc sulphate, copper sulphate and cobaltous acetate.

Ferolit was patented in 1994 [1].

Four wellknown elements regulate the all important functions in the organisms of sows and suckling-piglets. The metabolism of iron, copper, cobalt ant zinc acts as one unit in the organism of sows. If there is not enough iron in the blood, damage of zinc metabolism occur and toxicosis develops in the organism. Sows can fall ill with paraceratosis, the vitality of suckling-piglets decreases [14]. Cobalt takes part in the metabolism of vitamin B_{12}, in synthesis of hormones. The immunity and thermoregulating mechanisms of young suckling-piglets are very weak and the health of the piglets depends on microelements in the milk of the sows [18].

The aim of the work was:

- to determine the effect of different doses of Ferolit on the organism of sows and suckling-piglets;
- to investigate the penetration of iron through the digestive tract into the blood;
- to determine the action of the new composition on the morphological and biochemical blood tests;
- to carry out toxicological investigation on the organism of mice and rats.

Materials and methods

The preliminary investigations had shown that Ferolit could change the growth and haematological indexes of suckling-piglets if their mother got this composition. We determined the optimum doses of Ferolit. Then we carried out seven experiments and observed the effect of iron penetration in the organism of piglets and rabbits, investigated the morphological and biochemical indexes of blood, determined the doses *letalis toxica* of Ferolit in the organism of mice and carried out an experiment with rats. We observed the influence of Ferolit on diuresis and the functions of the liver and spleen. The blood was taken from ear and tail veins. Tests using fourty five day-old piglets and rabbits were done and the penetration of iron to blood through the digestive tract was observed. We used CBA mice (n=120) and Wistar rats (n=60) for the determination of the doses *letalis toxica*. Ferolit was given to rats in 1% of starch by probe and to mice in sunflower oil. Subsequently diuresis, quantity of chloride, protein in the urine and specific weight were determined. We evaluated the functional conditions of liver-alkaline phosphatase and pseudocholinesterase [38, 12].

The quantity of iron was measured using the biotest from the Czech Republic (Fe 70) [3]. Factions of protein were measured by the express method [5, 13]. The quantity of erythrocytes was determined with an erythrohaemometre and a Goriajev chamber, and of haemoglobin by the colour method and with a Sali haemometre. Trombocytes were measured with the Goriajev chamber [8, 13] and hematocrite with a Shkler microcentrifuge. The reserve of alkaline was determined by the Voltman–Klimaš method [5, 8] and the sedimentation of erythrocytes by the Panchenko method [8, 13].

We did the experiments in 1991—1994 in Lithuania. 13 Grams (650 mg Fe) of Ferolit were given to sows perorally three times on the 10th—14th days before parturition and on the 3rd, 10th—14th days after it. Ferolit was given to suckling-piglets on the 3rd and 8th days after birth through os. They got 2 grams of Ferolit which contained 100 mg of iron. The efficiency of Ferolit was compared with ferogliucin imported to Lithuania.

Results

Sows

Twenty three sows were used in seven experiments. We noticed that on the 8th day after parturition, the quantity of protein in the blood of the sows increased by 1—4% and the one of β-globulin decreased by 8.5% ($P<0.05$) under the influence of Ferolit [Table 1].

Table 1. *Changes of globulin fraction in the blood of sows under the influence of Ferolit.*

Group	Changes of fractions of globulin (%)	Before parturition	on the 8th day after parturition	on the 15th day after parturition
C	α	6.0±1.2	10.2±0.2	6.7±5.8
	β	28.1±16.7	13.2±3.2	6.2±9.2
	γ	47.5±5.5	70.6±7.9	77.1±9.4
F	α	4.3±2.5	9.3±5.8	11.0±0.1
	β	35.5±17.6	4.7±0.6	20.2±0.1
	γ	56.7±6.9	78.2±4.5	66.4±0.1

C — control; F — sows got Ferolit three times (650 mg Fe^3)=1950 mg Fe

After fifteen days the level of β-globulin was 14% ($P<0.01$) greater than that of control group. No difference in the reserve of alkali, quantity of trombocytes, hematocrite and sedimentation ratio of erythrocytes in the blood was observed in any of the groups. We used two forms of Ferolit solution 5-10-16-20-25 ml and powder, tried different doses and determined the optimum.

The best indexes of blood were obtained when the sows got 13 g (650 mg Fe) of Ferolit or suckling-piglets 2 g (100 mg Fe) through os [Figure 1].

Suckling-piglets

The piglets were divided into three groups and their weight was determined individually. The first group was kept as a control, suckling-piglets of the second group got 2 g of Ferolit (100 mg Fe) twice on the 3rd and 8th days after birth through os. Piglets of the third group did not get Ferolit, but their mothers got 13 g of Ferolit three times. The weight of the piglets of the third group increased by 23 g compared with the control, but there was no difference between the second group and control. The morphological and biochemical tests of piglets blood were done on the 8th, 15th and 28th days after birth and we determined that in the control group the quantity of haemoglobin was 98-86-96 g/l, in the second group 126-119-134 g/l and in the third one 117-119-118 g/l. We observed that when Ferolit was used in solution form, there were 83 g/l of haemoglobin, 5.00% of protein and 19.9 µmol/l of iron in the blood of piglets. When we used Ferolit in powder form there were 90 g/l of haemoglobin, 5.17% of protein and 20.6 µmol/l of iron. We decided that the latter form of the new composition was more effective. Eighty nine piglets were used in all experiments that were carried out in the autumn and spring. When Ferolit was included in the ration of sows, the level of haemoglobin was 1.2—4.8% and the amount of erythrocytes 7.2% greater compared with the control group of suckling-piglets. The transfer of iron from Ferolit into the blood was investigated with the six-week old piglets. We divided the piglets into two groups (five piglets in each) and observed their health for 24 hours. The piglets of one group got 6 ml (300 mg Fe) of Ferolit by probe. We took the blood after 1-3-6-12 and 24 hours after using Ferolit. The quantity of iron decreased after 1 and 3 hours, but after 24 hours there was 3.2% ($P<0.05$) more iron in comparison with the control group.

We also noticed that if only the sows got Ferolit, the quantity of haemoglobin of suckling-piglets increased by 1.8% ($P<0.05$) on the 8th day after birth, by 3.3% ($P<0.05$) on the 15th day and by 2.2% ($P<0.05$) compared with the control on the 29th day. If the suckling-piglets got Ferolit through os after 8-15-29 days level of haemoglobin was 2,7-3.3-3.8% ($P<0.05$) greater than that of the control group ($P<0.05$) [Figure 2].

Rabbits

Nine rabbits were divided into three groups. Rabbits in the first group were controls. We caused post-haemoragic anaemia in the animals of second and third groups. Then in one of these groups we used 1.5 ml (75 mg Fe) of Ferolit per os. The morphological and biochemical tests were done after 2-6-12-24 hours and 6 days. We determined that under the influence of Ferolit amount of the protein increased by 1.5% ($P<0.05$) and the quantity of iron increased after 24 hours. The blood indices of rabbits that did not get Ferolit were worse and did not achieve the level of physiological norm after six days.

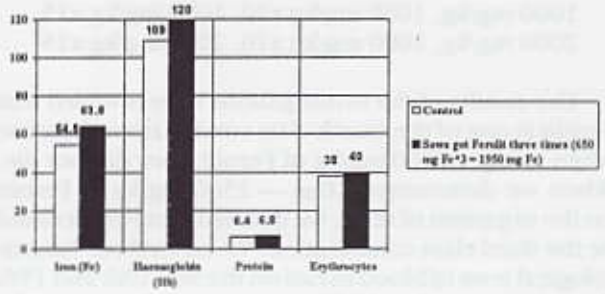


Figure 1. *Haematological indices of sows under the influence of Ferolit (indexes of 1-5-7 experiments). Iron (Fe) µmol/l, Haemoglobin (Hb) g/l, Protein %, Erythrocytes $x10^{12}$/l.*

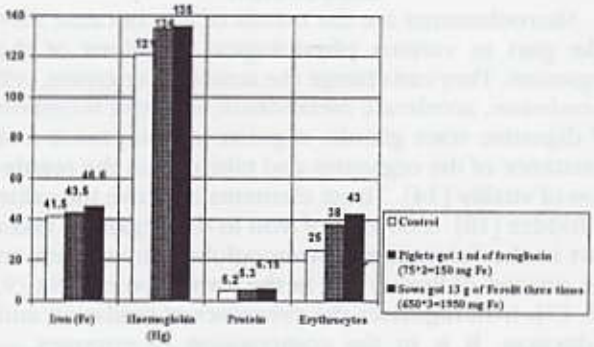


Figure 2. *Haematological indices of suckling-piglets under the influence of Ferolit and ferogliucin (age 21 days, indexes of 3—4 experiments).). Iron (Fe) µmol/l, Haemoglobin (Hb) g/l, Protein %, Erythrocytes $x10^{12}$/l.*

Toxicity of Ferolit

Experiments for Ferolit toxicity determination were carried out in Wistar rats and CBA mice. The rats and mice got Ferolit by probe, they had a normal appearance, did not die and their fur did not change. *Dosis letalis toxica* LD_{50} of Ferolit for the mice was 1500 mg/kg, but the rats got 7000 mg/kg of Ferolit. When the rats got 1000 mg/kg of Ferolit their weight was bigger by 18% or 36 g ($P<0.01$) than that of the control group, and it was bigger by (14% or 24 g ($P<0.05$) when they got 2500 mg/kg [Table 2].

Table 2. *Weight changes of rats under the influence of Ferolit.*

Doses of Ferolit mg/kg	Body weight (g)	
	after 10 doses	after 15 doses
1000	190±3.0	226±12.0
2500	170±2.0	194±11.0
Control	192±7.0	200±7.0

We have determined that Ferolit is not toxic after using it in a single dose. When we used Ferolit in 10 and 15 times larger doses there were no changes in the weight of the liver, spleen and kidney. The excretion of urine was of a normal level, the amount of protein and chloride did not change [8] and there was no damage in the function of the liver. The level of the quantity of alkali phosphatase and pseudocholinesterase was normal [2, 3, 12]. We used Ferolit in the following doses:

1000 mg/kg, 1000 mg/kg x10, 1000 mg/kg x15;
2000 mg/kg, 2000 mg/kg x10, 2000 mg/kg x15;

The results of the investigations have revealed that Ferolit is one of the fourth class combinations because when rats got 7000 mg/kg of Ferolit, they did not die. When we determined LD_{50} — 1500 mg/kg of Ferolit on the organism of mice, we decided that Ferolit could be the third class combination. We carried out haematological tests of blood in rats on the 5th, 10th and 15th days after using Ferolit. There was no difference in the level of erythrocytes and haemoglobin after using Ferolit in any of the groups. We did not notice an infringement of leukocytes.

Discussion

Microelements are the metals of life, because they take part in various physiological functions of the organism. They can change the activity of enzymes, cell membrane, accelerate metabolism, improve secretion of digestive tract glands, regulate development and resistance of the organism and take part in the regulation of vitality [14]. These elements improve the value of fodder [10]. 0.0065 % of iron in the organism takes part in the formation of haemoglobin, myoglobin, in the process of fission and in the formation of DNA [9, 10, 17]. Iron regulates the processes of oxidation and reduction. It is in the composition of enzymes — catalase, peroxidase, citochromoxidase.

The transfer place of iron into the blood is duodenum. The iron combines with alpopheritin and it is in the form of pheritin in blood. It can combine with β-globulin and form transpherine. One part of iron takes part in the formation of erythropoiesis and haemopoiesis, another part is reserved in the liver, kidneys and bone marrow, and the organism can use this reserve of it in the formation of haemoglobin [2, 6, 9].

The results of these investigations have shown that Ferolit improves haemopoiesis and erythropoiesis in the organism of sows and suckling-piglets. The quantity of haemoglobin and erythrocytes increases under the influence of the new composition. When the rabbits were ill with posthaemorrhagic anaemia, they got Ferolit and their physiological level of haemoglobin and erythrocytes achieved norm more quickly than in the control group. The transfer of iron from Ferolit into the blood took place in 24 hours. We noticed that in the blood of sows the level of β-globulin increased under the influence of Ferolit, so there was close contact between β-globulin and transpherin. The growth of the organism of rats was 14—18% faster after using various doses of Ferolit. Ferolit in the form of powder is more stable, easier to mix with fodder and its transfer into the blood is better than in the case of the solution. Ferolit can regulate the transfer of iron from haemoglobin of sows into the placenta and after birth of suckling-piglets haematological indices are better than those in the control group. We found more haemoglobin (90 g/l), iron (20.6 μmol/l) and protein (5.17%) in the blood when suckling-piglets got Ferolit through os. When only sows got Ferolit, there were 84 g/l of haemoglobin, 5.03% of protein and 19.9 μmol/l of iron in the blood of suckling-piglets. In many experiments the Ferolit was used in two ways. Sows got Ferolit with fodder and suckling-piglets got Ferolit per os. The second way is more complicated. The experiment with rabbits has shown that Ferolit regulates the action of bone marrow and there are more erythrocytes and haemoglobin in the blood after using it. The other experiment with mice and rats has shown that Ferolit is not toxic. The weight and function of the liver, spleen, kidney was normal. There was no damage in the forms of leukocytes. Ferolit has a positive effect on the growth of the organism. This new composition improves metabolism and the development and resistance of the organism of all the animals which were used in our experiments.

Conclusion

Generalising the results of our investigations we can conclude that Ferolit has a positive influence on the growth of the organism. The experimental piglets and sows had better morphological and biochemical blood indices. Ferolit is a new composition of microelements, it regulates haemopoiesis and erythropoiesis. The transfer of iron through the digestive tract into the blood serum increases under the influence of Ferolit. This composition had no negative influence on the organism of sows, suckling-piglets, rabbits, rats and mice. It was not toxic. We suggest that Ferolit be used for prophylaxis of anaemia and recommend that Ferolit is mixed with the fodder given to sows on the 10—14th days before parturition and on the 3rd, 10th and 14th days after it. The doses of Ferolit is 13 g (650 mg Fe) for sows. This composition can be used in 2 grams (100 g Fe) for suckling-piglets twice on the 3rd and 8th days after birth per os.

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Research Of New Veterinary Preparations For Prophylaxis And Treatment Of Mastitis In Cows

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Abstract

This article contains information about three veterinary preparations ("Mastistop", "Supermast" and "Optosept", created in the Lithuanian Veterinary Institute) designated for prophylaxis and the treatment of mastitis in cows. Treatment with "Mastistop" of 70 cows under the production conditions revealed that this preparation was most effective in cases of subclinical, serosial and catarrhal mastitis (89.4—100%). The lowest efficacy (60—64.3%) was observed while treating suppurative and fibrous inflammations of the udder. Tests on the preventive efficacy of "Supermast" revealed that injection of this preparation into the udder quarters after the last milking reduced the number of mastitis cases twice per three months if compared with the control group. The investigation data prove that treatment of the udder quarters with "Supermast" during the dry period makes it possible to reduce the number of mastitis cases during the dry period and lactation. The data on "Optosept" efficacy reveal that daily soaking of the udder and teats of 50 cows in this preparation after milking reduces the number of mastitis cases by 57.2% compared with the control group. Tests also revealed that the disinfection of teats with "Optosept" after milking reduces the number of mastitis cases during lactation.

Key words: Cow mastitis, treatment, prophylaxis, veterinary preparations.

Introduction

Great economic losses are induced by udder inflammation (mastitis) resulting in a deterioration of milk quality and technological characteristics and reducing the yield of milk. Such dairy cows are rejected as defective. While developing the treatment schemes and prevention measures it is necessary to achieve that preventive measures were used both during lactation and dry period. Investigations in various countries (1, 2, 3) revealed that mastitis prophylaxis in the dry period reduces the cases of udder inflammation, whereas treatment of udder quarters in this period with antibacterial preparations of extended effect allows the number of mastitis cases in the dry period and during lactation to reduce considerably, and reduces the preventive–treatment expenses.

The preparations injected prevent loss of milk yield and irritation of the mammary gland. Calved cows have mastitis less often and, therefore, the newborn calves have diseases of the alimentary tract only in rare cases. When lactation processes do not take place in the udder, antibacterial materials remain in it for a longer time, and therefore the efficacy of the treatment of chronic mastitis increases. During lactation it is necessary to keep to the rules of milking sanitation. Teat disinfection after milking is practised in many countries of the world (4, 5, 6). Disinfectant solutions cover the skin with a thin film and form a drop at teat end which prevents the microflora penetrating into the teat canal, reduces the number of pathogenic microbes, and prevents the udder skin from drying and chapping.

The treatment efficacy of udder inflammations depends on the volume and duration of the inflammation, quarter structure, resistance of the organism and udder, productivity of the cow, hygienic state, quality of the applied preparation, its dosage and the frequency of its application (7).

The aim of this work was to create and test three preparations for prophylaxis and treatment of mastitis in cows. "Mastistop" is applied during lactation. "Supermast"-during the dry period. "Optosept" is used for mastitis prophylaxis, i.e. disinfection of udder and teats after milking.

Methods

The composition of "Mastistop" includes furagin, rivanol, sulphadimesin, methyluracile and oil. "Mastistop" efficacy tests were conducted under experimental and field conditions. 8 cows were experimentally infected with *Staphylococcus aureus* and *Streptococcus agalactiae* cultures. The infective solution (2 bill. of microbe per 1 ml) was administered into the teat canal. After infection the cows were tested clinically, whereas their milk was tested in the laboratory. When signs of serous catarrhal mastitis of the udder were observed, 4 cows were treated with "Mastistop" and 4 with "Mastisan-A". The preparations were applied once per

Table 1. *Efficacy of "Matistop" in mastitis treatment during lactation.*

Preparation	Form of mastitis	Number of treated quarters	Number of applications	Number of cured quarters	Duration of treatment (days)	Efficacy of treatment (%)
Mastistop (dose 10 ml)	Catarrhal	20	4	18	3.8±0.2	90.0
	Serous	19	3	17	3.2±0.3	89.4
	Catarrhal-suppurative	12	7	9	6.9±0.2	64.3
	Fibrous	10	6	6	6.2±0.3	60.0
	Subclinical	25	2	25	2.5±0.1	100.0
Total:		89	22	76	—	85.4
Mastisan-A (dose 10 ml)	Catarrhal	20	4	17	4.0±0.3	85.0
	Serous	20	3	18	3.0±0.1	90.0
	Catarrhal-suppurative	12	8	7	7.2±0.3	58.3
	Fibrous	9	6	5	6.0±0.2	55.5
	Subclinical	26	2	26	2.6±0.1	100.0
Total:		87	23	73	—	83.9

day, 10 ml into a quarter, until the animal was cured. 70 cows were treated under field conditions (Table 1).

"Supermast" is an oil suspension of sulphalene, furagine and dibiomycine. The efficacy of the preparation was tested under field conditions. 20 cows which had a history of mastitis during lactation were used in the experiment. After the last milking 10 ml of the preparation was administered into each quarter of the udder. In the control group "Supermast" was not used. Every 15 days during the experiment the udder and secretion were tested clinically and in the laboratory (somatic cell count in milk). The experiment was continued till calving. All udder quarters were clinically controlled on 7th and 30th days after calving. Additionally milk samples were tested at the same time in the laboratory (somatic cell count in milk)."

"Optosept" is a combined preparation composed of phosphoric acid, Zinc sulphate, carbamide, glycerine, methylene blue and water. The preparation is designed for disinfecting the udder and teats after milking. The efficacy of "Optosept" was tested under field conditions. Before the application of the preparation, the udder quarters of all cows were tested clinically, and the milk in the laboratory. The cows were divided into two groups of 50. The udder and teats of cows in group 1 were soaked in "Optosept" solution after each milking. In the control group the preparation was not used. The tests lasted for three months. Every 15 days tests for mastitis were carried out. After three months, an analysis of the morbidity rate of both groups of cows was done.

Results

The results of these investigations revealed that after treatment of experimentally induced catarrhal mastitis all cows were cured in 5 days and after treatment with "Mastisan-A" in 6 days. The experiments carried out under field conditions showed (Table 1) that "Mastistop" is most efficient in the treatment of subclinical, serous and catarrhal mastitis (89.4—100%). The lowest efficacy was observed while treating suppurative and fibrous udder inflammations.

Table 2 shows that in the case of applying "Supermast" only two cows had cases of subclinical mastitis (10%) in three months. The number of clinical and subclinical mastitis cases in control group was how-

Table 2. *Cow mastitis morbidity rate.*

Group	Used in experiment		Had mastitis															
			During the 1st month				During the 2nd month				During the 1st month after caring				Total			
			Subclinical		Clinical		Subclinical		Clinical		Subclinical		Clinical		Cows		Quarters	
	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	No	%	No	%
C	20	80	2	4	1	2	1	2	0	0	2	5	1	2	7	35.0	15	18.7
E	20	80	1	1	0	0	0	0	0	0	1	2	0	0	2	10.0	3	3.75

C — control, E — experimental

Table 3. *Tests of preventive efficacy of "Optosept".*

Group	Used in experiment		Had mastitis															
			During the 1st month				During the 2nd month				During the 3rd month				Total			
			Subclinical		Clinical		Subclinical		Clinical		Subclinical		Clinical		Cows		Quarters	
	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	Cows	Quarter	No	%	No	%
E	50	20	1	2	0	0	1	1	0	0	1	2	0	0	3	6.0	5	2.5
C	20	200	3	5	1	2	1	2	0	0	1	1	1	1	7	14.0	11	5.5

C — control, E — experimental

ever 7 (35.0%). The data obtained reveal that the treatment of udder quarters in the dry periods with "Supermast" makes it possible to decrease considerably the number of mastitis cases during the dry period and lactation.

The investigations of the efficacy of "Optisept" revealed (Table 3) that after the udder and teats had been soaked in "Optosept", none of the 50 cows had clinical mastitis during three months, and only 3 cows had subclinical mastitis (6.0%). In the control group the number of clinical mastitis cases was 2, whereas the number of subclinical cases was 5. The total number of mastitis cases was 7 (14.0%). The rate of morbidity of the experimental cows, if compared with the control group, was lower by 57.2%. The data obtained show that teat disinfection after milking allows the number of mastitis cases during lactation to be reduced.

Conclusions

1. "Mastistop" was most efficient in the cases of subclinical, serous and catarrhal mastitis (89.4—100%).

2. Treatment of the udder and teats with "Supermast" during the dry periods allows to reduce twice the number of mastitis cases during the dry period and lactation to be halved.

3. Disinfection of the udder and teats with "Optosept" after milking reduces the number of mastitis cases by 57.2% if compared with the control gruop.

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CONTENTS

The Seroepidemiological Survey On The Spread Of Bovine Viral Diarrhea Virus (BVDV) In Estonian Cattle Herds — *A. Viltrop, J. Alaots, T. Laht, L. Ronsholt*

Bovine Leukosis In Lithuania — *J. Glamba, D. Apanavičiene*

Immunogenetic Aspects Of Resistance And Immunity — *Ü. Pavel, A. Viltrop*

Investigation Of Contagious Diseases In The Tartu Veterinary School And Tartu Veterinary Institute — *E. Ernits*

On The Mechanism Of Alimentary Acid-Base Balance Disorders In Cows — *K. Kadarik*

About Causes Of Pre- And Early Postnatal Mortality In Calves And Pathological Changes Found In Them — *N. Koslov, E. Lepp, V. Lokk, J. Song*

Ultrasound Guided Puncture Of Follicles In Heifers — *J. Kurykin, H. Gustafsson, B. Larsson, H. Rodriguez-Martinez*

Evaluation Of The Corpus Luteum Activity In Synchronized Recipients On The Day Of Embryo Transfer — *J. Kurykin*

Helminths Of The Most Important Game In Estonia — *T. Järvis*

Ultrasonic Measurement Of Animal Eye Dimensions — *M. Paunksniene, A. Paunksnis, V. Babrauskiene, A. Lukosevičius*

Isolation Of Canine Parvovirus In Cell Culture And Investigation Of Its Properties During Experimental Infection — *A. Stankevičius, A. Šalomskas, L. V. Belianko*

An Effect Of Ferolit On The Organism Of Animals — *J. Žymantiene, B. Girnius, Z. Beresnevičius, B. Sapijanskaite, K. Kraujelis, A. Ulinskaite*

Research Of New Veterinary Preparations For Prophylaxis And Treatment Of Mastitis In Cows — *V. Špakauskas*