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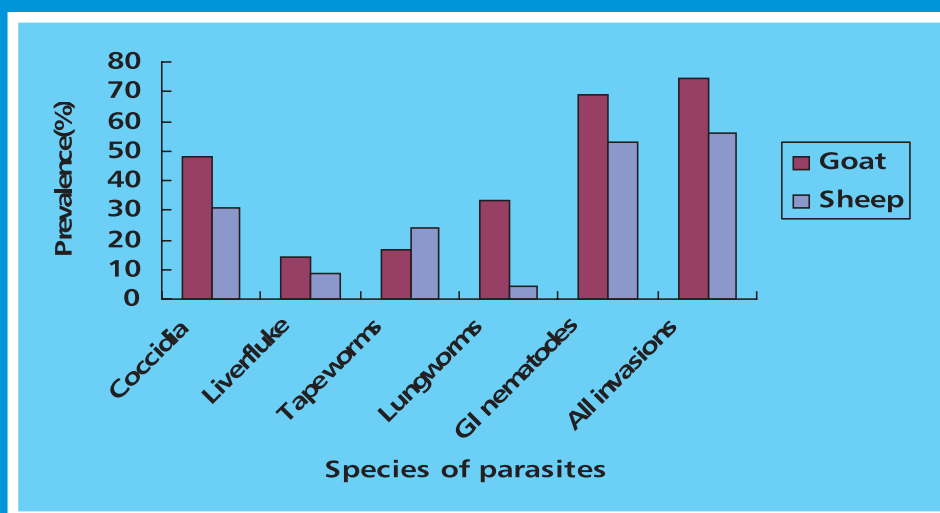


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SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK



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Address: Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

Naslov: Veterinarska fakulteta, Gerbičeva 60, 1000 Ljubljana, Slovenija

Tel.: +386 (0)1 47 79 100, 47 79 129, Fax: +386 (0)1 28 32 243

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REPRODUCTIVE PERFORMANCE AND VIABILITY OF NEWBORNS IN BUFFALOES TREATED ANTEPARTUM WITH VITASELEN AND/OR ULTRA-CORN

Hussein A. Amer*, Mohammed A. Hashem

Department of Theriogenology, Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

*Corresponding author, E-mail: amer_vet@hotmail.com

Summary: This study aimed to determine the efficacy of prepartum administration of Viteselen (VS) and Ultra-corn (UC) given at late gestation on IgG level, postpartum fertility, and viability of newborn calves. Forty five buffaloes were divided into 4 groups. First group served as control (n=9), while the others served as experimental groups (n=12/group). Second group received 30 ml of viteselen (1.7 mg sodium selenium and 150 mg vitamin E/ml). Third group was injected with ultra-corn (2 ml/100 Kg body weight). Fourth group was injected with viteselen and ultra-corn. The drugs were injected in 2 doses prior to anticipate date of parturition (60 days). The postpartum fertility was assessed. Colostrum from dams and serum from calves were collected to estimate the level of immunoglobulin (IgG). Body weight, growth rate and viability of calves were recorded. The periods required for fetal membrane expulsion and uterine involution were significantly ($P<0.05$) reduced in VS group when compared to UC or control groups. Concomitantly, injection of VS or UC significantly ($P<0.05$) reduced calving to first estrus interval and length of postpartum service period than control. The level of IgG was significantly higher ($P<0.05$) in both dam colostrum and newborn serum after administration of VS or VS+UC. Moreover, VS injection resulted in a significant ($P<0.05$) higher level of IgG in both dam colostrum (at parturition) and calves serum (at day-4 and -14 old) than the other groups. Only one case of abortion was received from VS+UC treated dams, while VS or UC treated dams delivered 100% healthy calves. The calves from treated dams showed higher body weight, growth rate/day and better vitality than control group. Likewise, the best body weight and growth rate/day obtained from VS treated buffaloes. No mortality in the 1st month occurred between newborns from VS+UC treated buffaloes compared to other groups. In conclusion, prepartum administration of VS or VS+UC appear to be beneficial, promising and improve the postpartum reproductive performance and calf viability in Egyptian buffaloes, and additional work involving a larger number of animals is suggested.

Key words: prepartum, buffaloes, viteselen, ultra-corn, IgG, calves, viability.

Introduction

A wide variety of antibacterial agents mainly antibiotics are employed for the prevention and treatment of infection in livestock, these antibiotics cause various problems such as residue and the emergence of bacterial resistance as well as suppression to the host resistance. Depressed immune function causes a marked increase in the incidence of oppor-

tunistic infection (1). Moreover, stress of pregnancy, parturition and lactation cause suppression of the host defense mechanism and increase susceptibility of the animal to infection during pregnancy, parturition and lactation (2).

Recently, there are many drugs used to increase the resistance of the animal by improving the humoral and cell mediate immune response such as Dihydroheptaprenol (3), vitamin B2 (4,5), selenium (6), vitamin E (7), vitamin E and selenium (8,9), and ultra-corn (10). Non-specific immunostimulants have been received considerable attention in the

vet-field. They appear to provide an efficient method of stimulating the immune system in a non-specific manner with few adverse side effects. Immunostimulating compounds have the potential to counteract the effect of environmental or microbial immunosuppressive factors. They may thus reduce morbidity and economic losses resulting from sub clinical or mild infectious diseases (11). Because they may also act as adjuvant, they can also potentiate immune responses to applied vaccine.

A great deal of interest has developed in implementing biosecurity programs to prevent the transmission of infectious disease to dairy replacement calves. One potential method of transmission of infectious diseases to dairy calves is through feeding infective colostrum and milk. The immune status of the newborn calves is dependent upon the passage of immunoglobulins from dams to the calves through the ingestion of colostrum (12) and its subsequent absorption from small intestine. Their net effect may therefore be to improve weight gain, and to lower mortality and morbidity rates among animals. The major immunostimulants are usually microbial preparations that are rapidly taken up by macrophages (13). Successful attempts to improve reproductive efficiency by administering immunopotentiators during late gestation in cows (14) and sows (15) have been reported previously. However, little information is available for buffaloes. The level of selenium and vitamin E in the soil and surrounding environment are neglected and we consider all the experimental animals are considered under the same standard condition. Thus, the present investigation aimed to evaluate the efficacy of some immunopotentiators (Viteselen and Ultra-corn) to improve the IgG-level, postpartum reproductive performance, as well as, viability of newborn calves in the Egyptian buffaloes.

Material and methods

Animals

This investigation was conducted on forty five buffaloes in the last trimester of gestation and belonging to a private farm at Sharkia Governorate, Egypt. The animals were 5.47 ± 1.14 years old, apparently healthy (weighted 485.92 ± 36.74) and free from common infectious and contagious diseases as proved by Veterinary Services Authorities. All the animals were received prophylactic routine treatment against internal, external and blood parasites.

The prophylactic immunization program included biannual vaccination against FMD, Rift Vally Fever and Hemorrhagic Septicemia. The last vaccination was given 4 months before the beginning of the experiment. All animals were dried off for 75 days prior to parturition. Each animal was supplied daily with 6 Kg concentrates, in addition to 25 – 30 Kg Barseem clover during the green season or darawa (green maize) during the dry season with suitable amount of rice straw.

Treatment schedule

The animals were randomly divided into one of four treatment groups. The first group served as the untreated controls (n=9). Another three groups served as experimental animals (each group included 12 buffaloes). Those in second group were injected i.m. with 30 ml of viteselen (1.7 mg sodium selenium and 150 mg vitamin E/ml) (Egyptian Co. for Chemical and Pharmaceutical-ADWIA, 10th of Ramadan City). Each buffalo in third group received 10 ml s.c. (2 ml/100 Kg body weight) ultra-corn which is a complete lysate of bacterial extract produced by ultrasound treatment of *Corynebacterium cutis*. (Virbac Co., France). In fourth group, the animals were injected with viteselen and ultracorn. The immunopotentiators were given 2 doses one week apart, the first injected on day 60 prior to the expected date of parturition.

Analysis of IgG level

Colostrum samples were collected within one hour of parturition (first milking) from buffaloes (at parturition) and at day-4 pp in all 5 groups. Blood samples were collected from newborn calves (at day-4 and day-14 old). Colostrum and serum samples were frozen at -20°C until determination of IgG. Frozen colostrum was submitted to determination of IgG level by using IgG Vet-RID (Radial Immunodiffusion) kit (Bethyl Laboratories, Inc., Montgomery, TX) Analysis was performed as previously reported (16). Due to the very high levels of IgG, colostrum samples were first diluted x10 with distilled water, and then 5 μl of the diluted sample was tested. This initial 10-fold dilution was taken into account when back-calculating colostrum IgG level for each sample. Serum IgG concentrations were determined using the same test kit and using the same general testing process. Serum IgG concentrations were determined according to kit instructions and using 5 μl of serum.

After the samples were placed on the plates, they were left at room temperature for a minimum of 18 h, and then the precipitation ring diameters measured and IgG values calculated. Three standards with known values (625, 2500, and 5000 mg/dl) were also tested for each run. The diameters of the known standards were then used to calculate the tested serum samples.

Reproductive performance

All the investigated buffaloes were kept under close observation during parturition and in the postpartum period until they were confirmed as pregnant again. After calving, the animals were kept individually in open pens and the period prior to the expulsion of the fetal membranes was recorded in hours. Rectal palpation was performed twice a week to examine the time of uterine involution (return to the non-pregnant size). Thereafter, estrus was confirmed in buffaloes by rectal palpation and they were inseminated using frozen thawed semen from a fertile bull.

Viability of the newborn calves

Calves born to the control and treated groups were weighed and kept under close observation. Their viability and health problems were examined

clinically and growth rate (Kg/day) was also recorded: (wt. at 3 months – wt. at calving)/90 days.

Statistical analysis

Data were analyzed using SAS analysis system package (17). Significant differences between each two means were evaluated utilizing Duncan's Multiple Range Test (DMRT) (18).

Results

The IgG level in colostrum of dams and serum of newborn calves were estimated (Table 1). Generally, the concentration of IgG was significantly ($P<0.05$) higher in the groups injected with immunopotentiators than control one. At parturition, the level of colostrum IgG was significantly ($P<0.05$) higher in the group injected with VS than the other test or control groups. At day-4 pp, the level of IgG was non-significant between the groups received immunostimulants but significant compared to the control one. Regarding the sera of newborn calves, they revealed significantly ($P<0.05$) higher level of IgG in the group administered VS than test or control group at day-4 old, and revealed a significant ($P<0.05$) higher level with VS and VS+UC injected groups than UC injected group at day-14 old.

Table 1: Mean (\pm S.E.) level (mg/ml) of immunoglobulin in colostrum of dams and serum of newborn calves after viteselen and/or ultra-corn antepartum administration

Parameters	Control group	Experimental groups		
		VS	UC	VS+UC
Parturient dams				
	N=9	N=12	N=12	N=12
At parturition	17.30±0.33 c	23.80±0.79 a	21.27±0.88 b	20.55±0.83 b
Day-4 postpartum	13.60±0.87 b	20.14±1.42 a	17.93±0.87 a	17.79±0.62 a
Newborn calves				
	N=7	N=12	N=12	N=11
Day-4 old	6.36±0.63 c	13.18±0.83 a	9.22±0.44 b	9.12±0.67 b
Day-14 old	4.18±0.57 c	10.59±0.87 a	6.28±0.75 bc	7.58±0.87 b

Means with different superscripts in each row are significantly different at level ($P<0.05$)
VS=Viteselen; UC=Ultra-corn

The mean values (\pm S.E.) of various parameters of pp reproductive performance of the buffaloes in four groups are illustrated in Table 2. The immunopotentiators appeared to improve the reproductive performance. Prepartum treatment with VS reduced

the period of fetal membrane expulsion compared to the other groups. Both of VS and VS+UC treatments had a significantly ($P<0.05$) shorter uterine involution period compared to the animals in the UC or control groups. Additionally, the VS and UC treated

groups had a significantly ($P<0.05$) shorter calving to 1st estrus interval and first service period compared to the control groups. There was insignificant number of services required per conception between the control and experimental groups.

The control dams delivered only 7 calves because there was one case of abortion and one case of stillbirth, while the group received VS+UC has one case of abortion. Meanwhile, VS and UC treated

dams delivered 12 healthy calves. The calves from treated dams showed higher body weight, growth rate/day and better vitality in comparison with the control. Likewise, body weight and growth rate/day were higher in newly born calves from VS than UC groups. Moreover, no mortality in the 1st month occurred between the newborn calves resulted from VS and UC treated buffaloes compared to the other groups.

Table 2: Mean (\pm S.E.) values of postpartum reproductive performance of buffaloes treated with viteselen and ultra-corn, and health status of their newborns

Items	Control group (n=9)	Experimental groups		
		VS (n=12)	UC (n=12)	VS+UC (n=12)
Postpartum reproductive parameters				
Placental expulsion (hr)	9.83±1.57 ^b	6.51±0.81 ^a	8.73±1.43 ^b	8.72±1.03 ^b
Uterine involution (days)	48.91±4.17 ^a	27.12±2.29 ^b	37.22±2.39 ^{ab}	34.23±7.29 ^{ab}
Calving to 1 st estrus (days)	185.11±28.17 ^a	110.72±15.45 ^b	113.11±17.01 ^b	167.50±22.90 ^{ab}
Service period (days)	225.31±33.03 ^a	143.71±16.49 ^b	153.76±13.08 ^b	210.79±42.60 ^{ab}
Services per conception	2.36±0.39 ^a	1.62±0.19 ^a	1.81±0.15 ^a	2.00±0.17 ^a
Health status of delivered calves				
Aborted feti	1	0	0	1
Still birth	1	0	0	0
BW at birth (kg)	34.7	38.3	34.5	35.9
BW 3 months pp (kg)	63.5	83.8	72.4	74.9
Growth rate (kg/day)	0.320	0.505	0.421	0.433
Mortality at 1 st month	2/9 (22.2%)	0/12 (0%)	0/12 (0%)	1/11 (9.1%)
Survival rate	7/9 (77.8%)	12/12 (100%)	12/12 (100%)	10/11 (90.9%)

Means with different superscripts in each row are significantly different at level ($P<0.05$)
 VS=Viteselen; UC=Ultra-corn; BW=Body Weight; pp=Postpartum

Discussion

Prepartum and early postpartum are critical periods for dairy animals. Proper nutrients intake in these periods helps in keeping the animal in a good condition and avoiding many problems. Vitamin-E and selenium (19) and ultra-corn (10,43,44) are essential nutrients for proper function of various reproductive characteristics of mammalian female. Moreover, pregnant animals are more susceptible to selenium deficiency than non-pregnant animals, which in turn increase the incidence of prepartum and postpartum reproductive disorders (20).

The level of IgG was significantly higher in both dam colostrum and newborn serum after administration of immunopotentiating agents than the control group. Moreover, viteselen injection resulted in a significant higher level of IgG in both dam colos-

trum and calves serum in comparison to the other test or control groups. However, the immune status of the newborn calves is dependent upon the passage of immunoglobulins from dams to the calves through the ingestion of colostrum (12) and its subsequent absorption from small intestine. When the mean colostrum-serum IgG levels immediately after birth and on Day 4 were evaluated, they were found to be higher in the experimental group than in the control group. When blood-serum IgG levels of the calves were measured, they were found to be higher after suckling colostrum (0-4 days after birth) than at 14 days after birth in the experimental group or the control group (21). On Day 4 and Day 14 after birth, IgG levels were found to be higher in the experimental group compared to the control group. Moreover, an important reason that calves have variable blood IgG at 24 hours of age is due to variation

in colostrum IgG content. Colostrum composition is remarkably variable, as colostral IgG can range from a low to high content (22). Subsequently, the amount of IgG in dam's colostrum depended mainly upon prepartum administration of immunopotentiators, and in calves depended mainly upon consumption of colostrum directly after parturition.

The significant shorter placental expulsion period in the viteselen treated buffaloes in the present study may be due to improved uterine muscular function. Both vitamin-E and selenium have antioxidant functions that protect biological systems from oxidative degradation (23-25). In addition to their general antioxidant roles, selenium and vitamin-E may be involved indirectly in prostaglandin synthesis where proxy radicals are a normal part of the metabolic pathways (24). Vitamin-E has been implicated in the control of Phospholipase-A2 activity (26), which is responsible for cleaving arachidonic acid from membrane phospholipids. Arachidonic acid is the common precursor for all prostaglandins and related compounds.

We expect that the levels of selenium and vitamin E in soil are very low and neglected; therefore we had positive influences on the reproductive performance after injection of the viteselen into the experimental animals.

The significant reduction in calving to first estrus and shorter service period in the buffaloes treated with VS compared with the control animals in the present study supported a previous study (27), who reported that the calving to the first estrus and the length of the service period were significantly reduced in cows treated with prepartum injection of vitamin-E and selenium. Contradictorily, after prepartum vitamin-E/selenium injection, there was no improvement on the subsequent postpartum reproductive performance of dairy cows (28). This discrepancy might be attributed to differences in the prepartum Se status of the animals and frequency of the injections (19). A significant decrease in the number of service per conception was obtained in the present investigation following prepartum VS injection. These findings reinforced those obtained by others (29,30). Additional studies have shown that supplementation with Se and vitamin-E reduces the incidence of retained placenta (29,31,32), metritis, cystic ovaries (29), clinical signs of mastitis (33), and time of uterine involution in cows with metritis (34). Selenium preferentially accumulates in the placentoms, ovary, pituitary and adrenal glands, suggesting specific requirements for Se in

those tissues (23,35). Several studies indicated that both humoral and cellular immune response are enhanced by vitamin-E/selenium treatment (27,36). However, a highly significant elevation of gamma globulins in the vitamin-E/selenium supplemented buffaloes compared to the control was noticed (37). Additionally, cows given vitamin-E and selenium in late pregnancy produce large quantities of colostrum and milk, and have less difficulty in drop of placenta (38).

Regarding preparatum ultra-corn (UC) treated group, a significant decrease was observed concerning uterine involution period, calving to first estrus interval as well as the service period, but still less than VS treated buffaloes. No available literature for the effect of preparatum treatment with UC on postpartum reproductive performance. However, ultra-corn is a complete lysate of bacterial extract produced by ultrasound treatment of *corynebacterium cutis*. The effect of UC on the chicken immune response to Newcastle disease virus vaccine was studied (10). They found that administration of UC induced marked effect on the immune response developed by Newcastle disease virus vaccine especially when given simultaneously with the vaccine. Administration of UC induced marked immunopotentiating effect in cattle, poultry and buffaloes via increasing phagocytosis, lymphocyte transformation index, total leukocytic count, lymphocytes, monocytes, antibody titre, immunoglobulin and gave protection against mortality (10,39,40). Some of these results are readily explicable on the basis of the expected effects of these immunostimulating corynebacteria. Thus killed corynebacteria (ultra-corn) are readily phagocytosed by macrophages and stimulate the release of tumor necrosis factor and inter-leukin I. These monokines may have a secondary effect on lymphocyte function and so stimulate the immune system non-specifically (40). Concomitantly, UC has several beneficial effects (41), where it stimulated phagocytosis; cell-mediated immunity; antibodies production and also it stimulated anti-virus defenses in increasing interferone production.

The control dams delivered only 9 calves because there was one case of abortion and one case of stillbirth. Meanwhile, VS and UC treated dams delivered 12 normal calves (in each group), except a case of abortion in VS+UC group. The calves from VS and treated dams showed higher body weight, growth rate/day and better vitality in comparison with the UC or control groups. Moreover, the control calves showed more severe pneumonia and

enteritis as a result of which 2 (22.2%) calves died within one month of birth. In contrast, the calves born to the preparatum treated dams showed mild disease symptoms and only one calf (9.1%) from UC treated dams died. The present results support the other views (10,40) who found that, UC treatment to late pregnant dams induced better state of delivery with no retained placenta or stillbirth in comparison with the control group. The newly born calves were of heavier body weight, better healthy status and highly resistance to disease. The pre and postpartum supplementation with vitamin-E/selenium combination improved the reproductive efficiency and immune status of Egyptian buffaloes and resulted in a significant elevation of gamma globulins (37). Consequently, a high immunoglobulin-G concentration was observed in calves supplemented with vit.E/Se (42). Circulating IgG has been related to preweaning growth (43) as well as long term performance of calves (44), thus some commercial calf raisers will pay dairy producers a premium for providing calves with serum total protein that exceeds some critical threshold (usually >5.2 to 5.5 g of total protein/dl of serum). Others will reduce the amount they pay to the producer if total protein is too low. Although passive immunity has an important effect on calf health, there are a number of other factors that influence the overall cost of morbidity and mortality on a calf raising operation. These other factors include the level of exposure of calves and level of stress to which calves are exposed. Another critical control point during the calf's life is the first 24 hours. Consumption of colostrum is essential to provide animals with the antibodies and other proteins that calves need to stay healthy. The amount of colostrum (and immunoglobulin, or IgG) consumed determines amount of passive immunity and resistance to disease. When calves consume insufficient amounts of IgG from colostrum within the first 24 hours of life, they are much more susceptible to developing disease and possibly dying. A major reason that preweaning mortality is higher than optimum (defined as less than 5% of calves born alive) is due to inadequate IgG intake (22). Measuring a calf's level of passive immunity within the first week of life allows the producer to know the effectiveness of the colostrum management and calf feeding program. Because this is so important to the health and survival of the calf, it is an essential part of monitoring the overall heifer operation. However, the importance of achieving adequate levels of colostral immunoglobulins to protect the neonate from enter-

ic disease and septicemia has long been recognized (45).

It would be anticipated that as a result of increased health, the newly born calves would show enhanced weight gain. The effect on newborn calves is somewhat more difficult to understand. Clearly, the killed bacteria given to pregnant cows will effectively stimulate their macrophage function and the release of cytokines. These cytokines are relatively small proteins (with molecular weights on the order of 20-30 Kda). Proteins of this size may readily cross the placenta. Thus, any immunostimulating effect on the dam should also be effective in the fetus. Calves born to treated buffaloes may thus be at a significant advantage over untreated calves in the face of microbial challenge (in ultra-corn group). Stimulation of non-specific defense mechanisms has the potential to counteract at types of immunosuppressive effects in animals. This is especially true in a country such as Egypt, where there is a high level of sub clinical diseases in reared animals. Sub clinical virus infections, suboptimal nutrition, intestinal parasitism, and so on may all result in mild immunosuppression.

In conclusion, the buffaloes have been criticized for its poor reproductive performance. Thus, it appears that a positive effect could be obtained by prepartum immuno-stimulation using viteselen, ultra-corn or combination on postpartum reproductive efficacy and viability of newborn calves. Additional work involving a larger number of animals is suggested to reach appropriate results.

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REPRODUKCIJSKA SPOSOBNOST IN VITALNOST NOVOROJENCEV PRI BIVOLIH, TRETIRANIH S PRIPRAVKOMA VITESLEN IN ULTRA-CORN V PREDPORODNEM OBDOBJU

H. A. Amer in M. A. Hashem

Povzetek: V raziskavi smo želeli ugotoviti vpliv pripravkov viteselen (VS) in ultra-corn (UC) na raven imunoglobulinov G, na poporodno plodnost in vitalnost novorojencev. Imeli smo 45 bivoljih samic, ki smo jih razvrstili v 4 skupine. Prva (n=9) je bila kontrolna, ostale pa eksperimentalne (n=12/skupino). Živali druge skupine so prejele po 30 ml viteselena (1,7 mg sodium selenium in 150 mg vitamina E/ml). Živalim tretje skupine smo aplicirali ultra-corn (2 ml/100 kg telesne mase). Živalim četrte skupine smo dali oba pripravka. Pripravka smo vbrizgali v dveh odmerkih 60 dni pred pričakovanim dnevom poroda. Po porodu smo ugotavljali telesno maso, prirastek in vitalnost telet, ocenjevali smo reprodukcijsko sposobnost mater ter v mlezu in serumu telet določili raven IgG. Pri skupini, ki je prejela VS, je bil čas luščenja posteljice in involucije maternice bistveno krajši kot pri UC in kontrolni skupini ($P<0.05$). Je pa aplikacija katerega koli od pripravkov bistveno skrajšala poporodni premor do prve gonitve in poporodni premor do zabeležitve ($P<0.05$). Raven IgG je bila bistveno večja ($P<0.05$) tako v mlezu mater kot v serumu novorojencev pri dajanju VS ali VS+UC. Dodajanje VS je vplivalo na bistveno višjo raven IgG tudi še pri štiridnevni in dvotedenski teletih. Pri eni od živali, tretirani z VS+UC, je prišlo do abortusa, ostale iz vseh tretiranih skupin pa so povrgle popolnoma zdrava teleta. Teleta tretiranih mater so imela višjo povprečno telesno maso, večji dnevni prirastek in boljšo vitalnost kot teleta iz kontrolne skupine. Najboljši prirastek smo zabeležili pri teletih iz skupine VS. V prvem mesecu po rojstvu ni poginilo nobeno tele. Zaključimo lahko, da predporodno dajanje pripravka viteselen ali kombinacije viteselena in ultra-corna pozitivno vpliva na poporodno reprodukcijsko obdobje in vitalnost telet egiptovskih bivolov, za natančnejšo opredelitev pozitivnih učinkov pa bi bile potrebne raziskave na večjem številu živali.

Ključne besede: predporodno obdobje; bivoli; teleta; viteselen; ultra-corn; imunoglobulini G; vitalnost

ANTIOXIDATIVE EFFECT OF OREGANO SUPPLEMENTED TO BROILERS ON OXIDATIVE STABILITY OF POULTRY MEAT

Slavomír Marcinčák*, Rudolf Cabadaj, Peter Popelka, Lýdia Šoltýsová

Department of Food Hygiene and Technology, University of Veterinary Medicine, Komenského 73, 041 01, Košice, The Slovak Republic

*Corresponding author, E-mail: marcincak@uvm.sk

Summary: Effect of oil extract of oregano supplemented in diet, on growth and oxidative stability of poultry meat was studied. Broiler chickens fed with addition of oregano achieved higher weight (2563 ± 140 g) in comparison with control group (2462 ± 195 g). Oxidative processes were investigated as changes of malondialdehyde content in breast and thigh meat after 0, 3, 6, and 12 months of storage at -21 °C. Partition of defrost samples was stored at chilling conditions (4 °C) during 12 hours, ground and thermally treated at 80 °C during 15 minutes, to observe antioxidative effect of added oregano oil in poultry meat after thermal treatment. Results showed that oregano essential oil was more effective in delaying lipid oxidation compared to the control diet at all time points. Thigh meat was more susceptible to lipid oxidation compared to breast meat ($P < 0.05$). Same effects of oregano extract were observed in meat after warm heating ($P < 0.05$).

Key words: broiler performance; lipid oxidation; oregano essential oils

Introduction

Poultry meat has some advantages from nutritive aspect e.g. the high content of proteins, essential polyunsaturated fatty acids, minerals and the low content of lipids (1). At average broilers have from 3.5 to 5.0 % of fatty tissues (18). Poultry fat contain higher amount of polyunsaturated fatty acids (PUFA) than fatty tissues of other slaughtered animals. Exactly, PUFA are the most sensible fractions to oxidation processes. Lipid oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavours and changes in the colour of meat (5, 11).

Antioxidants are applied to protect food and avoid oxidation processes. Antioxidants are defined as substances which occur in meat in very low concentrations in comparison with substrate sensible to oxidation and they inhibit or reduce its oxidation (2, 13, 16).

Important source of natural antioxidants are plants. Rosemary (*Rosmarinus officinalis* L.) and sage

(*Salvia officinalis* L.) were recognized as plants with the highest antioxidation activity by many authors (4, 5, 10, 14). Thyme (*Thymus serpyllum vulgaris* L.) and marjoram *Origanum majorana* L.) have also significant antioxidation effect and they reduce autooxidation of fats (3).

Oregano (*Origanum vulgare* L.), is an aromatic plant with a wide distribution throughout the Mediterranean area. The essential oil from oregano obtained by steam-distillation process from leaves and flowers is well known for its antioxidative activity (7). Carvacol and thymol two main phenols that constitute about 78 – 82 % of the essential oil of oregano are principally responsible for this activity. The essential oil derived from oregano is known to possess also antimicrobial, antifungal, and insecticidal activities. Antimicrobial activity of oregano has been examined as an alternative grow promoter in broiler chicken (6), broiler turkeys (17) and pigs.

A high oxidative stability of meat is important when attempting to avoid or delay development of rancid products or warmed-over flavour. In relation to character of process of lipid oxidation, effect of antioxidants is the more significant, the sooner they are applied. Ideal situation is the fats are protected immediately after slaughtering of animals (8,

13). This protection can be achieved due to feeding of antioxidants in live animals (12). Increased antioxidative status in the living animal and the following increased oxidative stability of the raw product is considered beneficial the consumer and processing industry.

The aim of our experiment was observation of supplementation of feedstuff with oil extract of oregano on growth and oxidation stability of frozen and thermally treated poultry meat.

Materials and methods

Animals and diets. Experiment was carried out with 60 one-day-old ROSS 308 broiler chickens. Broilers were stabled in approved animal quarters in I. Internal clinic of University of Veterinary Medicine in Košice and they were divided into two groups: First group (EO) was fed with standard diet supple-

mented with oil extract of oregano (*Calendula Nová Ľubovňa*, Slovak republic) in concentration 0.05 % per 1 kg. Essential oil of oregano, on rice substrate, was mixed to expected daily dose of standard diet every day. Control group (K) was fed with standard diet without supplementation of antioxidants. Feed and water were provided ad libitum during the 42 day on study.

Temperature was gradually decreased from 33 °C on day 1 to 22 °C on day 21 and then kept constant. The lighting regimen provided 24 h of continuous light per day. The humidity of environment was 70 %. All chickens were individually weighted on the 1, 7, 14, 21, 28, 35, and 42 day of age. Animal handling was according to the Guidelines established by the European Union on Animal Care (Council Directive 86/609) as required by the ethical committee of the University of Veterinary Medicine in Košice (Slovak republic).

Table 1: Composition of standard diet

Components	g.kg ⁻¹ feed	Chemical analysis	g.kg ⁻¹ feed
Corn	590	Dry matter	860
Soybean meal	254	Crude protein	210
Full fat Soybean	65	Crude fibre	35
Wheat	11	Ash	70
Fish meal	25		
Yeast	25	Calculated analysis	
Limestone	15	Linoleic acid	10.0
DL-Methionine	2	Calcium	8.0
Monocalcium phosphate	8	Phosphorus (total)	6.0
Sodium chloride	3	Lysine	11.0
Vitamin premix ¹	1	Methionine+Cystine	7.5
Trace-mineral premix ²	1	Metabolizable energy MJ.kg ⁻¹	12.0

¹ Supplying per kg diet: vit. A, min. 10000 IU; vit. D3, min. 2000 IU; vit. E, 25 mg; vit. B2, min. 4 mg; vit B12 20 µg; folic acid, 1 mg. ²Supplying per kg diet: Zn, 50 mg; Mn, 50 mg; Fe, 60 mg; Cu, 6.0 mg; Se, 0.75 mg

Processing of chickens. On the 42nd day of age broiler chickens were slaughtered. Bleeding of chickens followed after stunning in respect of rules established for slaughtering of animals and it was performed by responsible veterinary surgeon. Afterwards, all poultry carcasses were weighted, deboned, skin was removed and chilled (4 °C). Packed samples of breast and thigh muscles (in polyethylene sacks) were stored in freezer at – 21 °C during 3, 6 and 12 months. Samples were defrost at chilling conditions (4 °C) for the duration of 12 hours before analysis

and homogenized in grinder.

Partition of defrost samples (10 pieces) taken from both groups was stored at chilling conditions (4 °C) during 12 hours, ground (Ø 4,5 mm) and thermally treated at 80 °C during 15 minutes, to determine antioxidative effect of added oregano oil in poultry meat after thermal treatment.

Evaluation of Thiobarbituric acid assay. Decomposition of fats is assessed by the thiobarbituric acid (TBA) assay. This test is simple, fast and it is based on the reaction of 1 molecule MDA with 2 molecules

of TBA (15). The color of the final complex is pink and the absorbance of the complex is measured spectrophotometrically. MDA is major degradation product of oxidation of polyunsaturated fatty acids. Evaluation of TBA was performed according to Marcinčák et al. (14) and measured spectrophotometrically at 532 nm (Helios Y, v. 4.6, Thermo spectronic, Great Britain).

Statistical analysis. Statistical processing of results was performed by Graph Pad Prism 3.0 (1999). Results are expressed as arithmetic mean (x) and standard deviation (sd). Comparison of results among groups was statistically evaluated by Student t-test. Advanced growth of malondialdehyde in both groups during storage was compared by one-way ANOVA test. Tukey comparison test was used to compare statistical differences between values and $P < 0.05$ was considering as statistically significant difference.

Table 2: Average weight of broilers during feeding period

	Day of feeding						
	1.	7.	14.	21.	28.	35.	42.
K	64 ± 7	192 ± 22	353 ± 29	696 ± 74	1242 ± 118	1944 ± 210	2462 ± 195
EO	68 ± 6	215 ± 17	409 ± 28	766 ± 65	1326 ± 104	1972 ± 142	2563 ± 140

In table 3 are expressed results of determination of TBA value in meat samples stored in freezer at -21 °C. Immediately after slaughter processing, concentration of MDA was slightly lower in breast muscles of broilers from group EO than in control group ($P > 0.05$). Storage of samples in freezer (-21 °C) for 3 months caused moderate growth of MDA in control group ($P > 0.05$). MDA values in samples obtained from broilers supplemented with oil extract of oregano remained stable during this period. Significant growth of MDA concentration in samples of breast muscles became obvious in both groups within 6 months of storage at freezing conditions (-21 °C). Growth was more significant in control

Results

Table 2 shows average weights during feeding period in both groups of broilers. Addition of oil extract of oregano in diet was manifested within 7 days of feeding and for the duration of feeding broilers from group EO achieved higher weight than control group (K). At the end of feeding period (42nd day), average weight of broilers supplemented with extract of oregano was 2563 g in comparison with control group 2462 g. However, weight differences between groups were not statistically significant ($P > 0.05$). No differences in feed intake and feed : gain ratio were observed in broilers fed with oregano essential oil in comparison with control (data not presented).

group (0.184 ± 0.018 mg.kg⁻¹) in comparison with EO group ($0.138 \pm$ mg.kg⁻¹), what indicates higher oxidation stability of poultry meat after addition of oil extract of oregano ($P < 0.05$). The same phenomena was observed within 12 months of storage.

Immediately after slaughter processing, MDA concentrations in thigh muscles were higher than in breast muscles in both groups ($P < 0.05$) and MDA concentration in thigh muscles from control group was significantly higher than in EO group ($P < 0.05$). The same data were determined also within storage of samples for 3, 6 a 12 months in freezer at -21 °C ($P < 0.05$).

Table 3: Oxidation changes of fats expressed as amount of malondialdehyde (mg.kg⁻¹) during storage of samples in freezer (-21 °C)

		Storage (months)			
		0	3	6	12
K	Breast	0.140 ± 0.014	0.151 ± 0.015	0.184 ± 0.018	0.496 ± 0.088
	Thigh	0.235 ± 0.020	0.237 ± 0.010	0.272 ± 0.021	0.652 ± 0.102
EO	Breast	0.121 ± 0.009	0.122 ± 0.011	0.138 ± 0.013	0.311 ± 0.098
	Thigh	0.177 ± 0.013	0.181 ± 0.007	0.197 ± 0.009	0.465 ± 0.072

Table 4: Oxidation changes of fats expressed as amount of malondialdehyde (mg.kg⁻¹) after thermal treatment (80 °C, 15 min).

		Storage (months)			
		0	3	6	12
K	Breast	0.24 ± 0.06	0.38 ± 0.07	0.46 ± 0.14	1.51 ± 0.21
	Thigh	0.59 ± 0.13	0.66 ± 0.15	0.94 ± 0.21	2.35 ± 0.27
EO	Breast	0.19 ± 0.04	0.22 ± 0.05	0.31 ± 0.17	0.79 ± 0.17
	Thigh	0.34 ± 0.07	0.48 ± 0.007	0.54 ± 0.14	1.72 ± 0.14

Table 4 shows results of determination of TBA value in breast and thigh muscles from both groups after thermal treatment of samples. Thermal treatment accelerated oxidation processes in both groups in comparison with untreated samples ($P < 0.05$). Following storage and thermal treatment increased MDA concentrations as a consequence of accelerated oxidation processes. Addition of oil extract of oregano had positive effect on delaying of oxidation of fats in comparison with control samples ($P < 0.05$). Thigh meat was again more susceptible to lipid oxidation compared to breast meat.

Discussion

Obtained results indicated that addition of oregano in dose 0.05 % per kg of feed gently increased the slaughter weight of chicken. These results correspond with data (6, 14, 19), which indicate moderate higher weight of broilers after feeding of plant extracts. Plant extracts effects may be due to the greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence to suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects (9).

Poultry meat contains less amount of fat than red meat of slaughter animals. On average, broilers contain from 3.5 to 5.0 % of fat. Poultry fat has higher amount of unsaturated fatty acids (PUFA) than fat of other slaughter animals. Regarding higher amount of PUFA, poultry meat is more sensitive to oxidative processes (17). Feeding and conditions used for breeding and slaughtering can influence oxidative stability of meat (2, 8). The most ideal situation is when fats are protected immediately they are derived. It means before slaughtering of animals. It is possible gain this fact when tissues are saturated with antioxidants as an additives during the life of animals (6, 13). Govaris et al. (8) stated that, post-mortem addition of antioxidants to the mince meat

also retarded lipid oxidation in the prepared patties compared to control; however, this effect was inferior to that of dietary supplementation even though the post-mortem α -tocopherol supplemented meat contained 90-fold more α -tocopherol than patties from the dietary supplemented meat.

Immediately after slaughter processing, MDA concentrations were low in both groups. However, following storage caused growth of MDA in samples. Obtained results indicate that addition of antioxidants had significant effect on reducing of oxidation processes in meat. Our results confirmed that thigh meat samples suffered more intensive lipid oxidation than breast meat samples, throughout the 12 months storage period. This is generally in agreement with other research studies that have investigated the effects of oregano essential oils in meat protection from oxidation through feeding (5, 6, 8, 17). Thigh meat was more susceptible to lipid oxidation compared to breast meat. The greater susceptibility of thigh to lipid oxidation has been attributed to the higher content of PUFA in this tissue (15, 17).

Process of production of meat products (cutting, grinding, and mixing) causes degradation of muscle membrane system and has a strong influence on oxidation of intracellular fat, primarily phospholipids (2). In thermally treated meat products level of oxidation depends on intensity of thermal treatment. Thermal treatment accelerated oxidation expressed in both groups as high TBA values in comparison with untreated samples ($P < 0.05$). Addition of oil extract of oregano had positive effect on delaying of oxidation of fats in comparison with control samples ($P < 0.05$). Consistently with our results, Florou-Paneri et al. (6) reported that both the oregano herb and essential oil were effective in delaying lipid oxidation of breast and thigh meat during refrigerated storage, when they were dietary supplemented to turkeys. Similar results were described by Govaris et al.(8), who reported that addition of oregano oil in feed of turkeys and post mortem in ground meat increases stability of fat oxidation.

The lower MDA values found in tissues after diet supplementation with oregano, are probably the results of various antioxidants constituents that entered the circulatory system, distributed and retained in the tissues, exhibiting antioxidant activity (6)

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ANTIOKSIDATIVNI UČINEK ORIGANA KOT PREHRANSKEGA DODATKA BROJLERJEM NA OKSIDATIVNO STABILNOST PIŠČANČJEGA MESA

S. Marcinčák, R. Cabadaj, P. Popelka, L. Šoltýsová

Povzetek: Preiskali smo učinek oljnega ekstrakta origana na prirastek piščancev in oksidativno stabilnost njihovega mesa. Brojlerji, ki smo jih krmili z origanom kot prehranskim dodatkom so dosegali večjo telesno maso (2563 ± 140 g) v primerjavi s kontrolno skupino (2462 ± 195 g). Oksidativni proces v mesu smo spremljali z ugotavljanjem vsebnosti malondialdehida v prsnem fileju in stegenskem mesu na začetku shranjevanja pri -21°C ter po treh, šestih in dvanajstih mesecih. Da bi ugotovili antioksidativni učinek origana po termični obdelavi, smo koščke odmrznjenega mesa do 12 ur hranili pri 4°C in jih termično obdelovali pri 80°C petnajst minut. Rezultati kažejo, da esencialno olje origana učinkovito zadržuje oksidacijo maščob v zamrznjenem mesu v vseh preiskanih časovnih obdobjih. Stegansko meso je bolj podvrženo oksidaciji maščob, kot pa prsi ($P < 0,05$). Enak učinek ekstrakta origana je bil ugotovljen v mesu tudi po termični obdelavi ($P < 0.05$).

Ključne besede: brojlerji; prirastek; oksidacija maščob; esencialno olje - origano

HELMINTHIC AND PROTOZOAN INTERNAL PARASITIC INFECTIONS IN FREE RANGING SMALL RUMINANTS OF BANGLADESH

K. B. M. Saiful Islam*, M. J. F. A. Taimur

Animal Health Research Division, Bangladesh Livestock Research Institute, Savar-1341, Dhaka, Bangladesh

*Corresponding author, E-mail: vetkbm@yahoo.com

Summary: A year-round study was carried out on 136 Bengal sheep and 224 Bengal goats with the aim to compare the species diversity and prevalence of infections with protozoa, flukes, tapeworms and nematodes parasitizing gastrointestinal tract and lungs of the small ruminants from various parts of Bangladesh. The prevalence of internal parasitic infections was higher in goats (74.55%) than in sheep (55.88%). Liver fluke (*F. gigantica*) was more prevalent in goat (14.28 %) than in sheep (8.82%) whereas tapeworm infection was more frequent in sheep (24.26%) in comparison to goat (16.52%). In addition, goats showed higher than sheep prevalence of protozoan (*Eimeria spp.*) and lungworm (*Muellerius spp.*) infections. Goats (33.48%) showed eight times higher prevalence of *Muellerius capillaris* (lungworm) infections than sheep (4.41%) did. Lungworm infection was more likely to occur in female goats where as other species were more prevalent in male. The most prevalent gastrointestinal nematode in both host species was *Trichostrongylus* followed by the occurrence of *Haemonchus*. A total of 10 different types of internal parasites were identified of which 9 were common for both species. The most commonly occurring parasites in both species include *Eimeria*, *Trichostrongylus*, *Haemonchus*, *Monizia* and *Fasciola*.

Key words: helminth; protozoa; parasitic; prevalence; species diversity; sheep; goat

Introduction

There are about 38.1 million small ruminants (goat and sheep) in Bangladesh (1) which plays an important role in the rural economy and earn substantial amount of foreign currency by exporting skins and other by-products (2). Goat production is becoming more and more popular in Bangladesh and nowadays special emphasis is also given to the raising of sheep. Helminthiasis, specially parasitic gastro-enteritis (PGE) constitutes a serious health problem and limitation to the productivity of small ruminants throughout the world due to the associated morbidity, mortality and cost of treatment and control measures (3). In Bangladesh parasitism has been considered as one of the major constraints of livestock production (4). The incidence of para-

sitic diseases is usually very high and ranges from 30–80% in ruminants and the young crossbred animals are severely affected (5). Losses arise from deaths (10–15%) of young animals, stunted growth, reduced milk and meat production and draft output, delayed maturity and prolonged calving intervals (5). Goats and sheep have numerous gastrointestinal parasites, many of which are shared by both species. The most important include coccidia (protozoa), nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). Gastrointestinal nematodes of *Trichostrongylidae* family are perhaps the most important parasites of small ruminants world-wide, causing significant morbidity and loss of production.

Owing to the growing demand for high quality animal proteins for human consumption, small ruminants occupy a special place as they are extremely efficient in converting the indigestible cellulose and hemicellulose to animal protein. As goat

and sheep rearing in Bangladesh being popular with days because these species are valuable for economic, managerial and biological reasons, special emphasis has to be employed regarding proper health & production of them. Among the multitude of problems hindering the livestock development in Bangladesh, disease problems specially related to endoparasitism constitute a serious threat to the successful small ruminants' industry. Despite the special emphasis on the rearing small ruminants, the development of the industry in Bangladesh is seriously threatened.

So, it is essential to know the type of parasites involved in the production of parasitic diseases in goat and sheep industry for its treatment, prevention and control under field condition. Though, some works have been reported on the parasitism of livestock in Bangladesh, no precise report has been found on the prevalence and distribution of helminthic as well as protozoan parasites of goat and sheep together. To the best of our knowledge, this would be the first time report on helminthic and protozoan parasites of goat and sheep. The principal aim of this work was to investigate prevalence of internal parasites of goats and sheep in Bangladesh and to identify species diversity of helminthes in goats and sheep so that proper measure could be taken to help prevent and control the small ruminant industry from the devastating effect of parasitism accordingly.

Materials and methods

Animals and collection of samples

The study was carried out for one year from March 2005 to February 2006 on 136 indigenous Bengal sheep and 224 black Bengal goats from different parts of Bangladesh. The animals were reared under free ranging system where they were allowed for grazing on nearby pasture. Only concentrate was supplied once or twice daily. Faecal samples were collected randomly from the rectum of animals in individual sterile polythene bag and carried on ice to the laboratory and either used promptly or stored at 4°C for a maximum of 24 hours.

Parasitological procedures

Prevalence of gastrointestinal parasites was estimated using flotation in saturated NaCl and sedimentation methods. In order to assess species of gastrointestinal nematodes, infective (L3) larvae were cultured using samples of faeces from individual animals. Faecal samples weighing between 2 and 5 g from each goat or sheep in the study were prepared into a faecal culture for third stage larvae development (6). After 7 days of incubation larvae from faecal cultures were harvested and identified to genera or species using light microscope. The differential counts and identification of each nematode species were performed according to the descriptions of Soulsby (7) and Hansen and Perry (8). Baermann's technique was used for detection of lungworm infection.

Results

As many as 74.55% of examined goats was infected with at least one genus/species of parasite. The prevalence of parasitic infections was higher in goats than in sheep (55.88%) reared in the same conditions (Fig. 1).

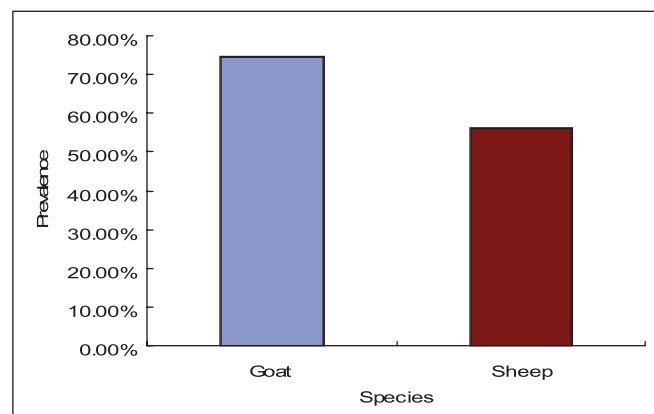


Figure 1: Overall prevalence of parasitic infections in goat and sheep

Liver fluke (*F. gigantica*) was more prevalent in goat (with prevalence 14.28%) whereas tapeworm infection was more frequent in sheep (24.26%) (Fig.2). In addition, goats showed higher than sheep prevalence of *Eimeria* spp. and *Muellerius* spp. (lungworm) infections (Fig.2). With the later, goats were about 8 times more frequently infected than sheep.

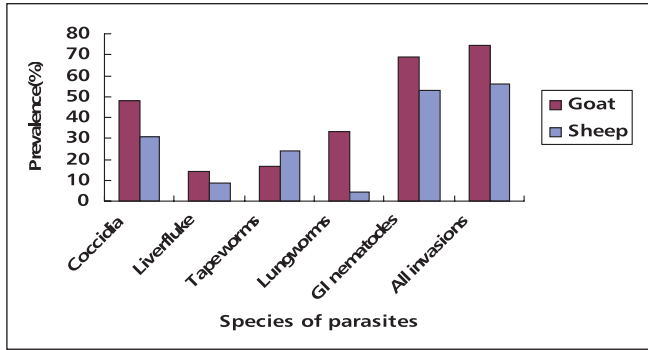


Figure 2: Prevalence of protozoa, trematode, tapeworms and nematode infection in goats and sheep in Bangladesh

Female goats seem to be more susceptible to infections with lungworms and males with the rest of genus/species but the overall infection was higher in females (Fig-3).

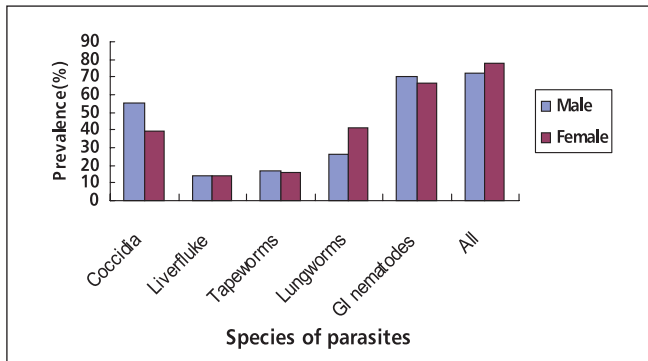


Figure 3: Extensiveness of parasitic infections in males and females of goats

Table 1: Species diversity of internal parasites in goats and sheep in Bangladesh

Parasites	Goat % positive (n=224)	Sheep % positive (n=136)
<i>Eimeria spp.</i>	47.76	30.88
<i>Fasciola gigantica</i>	14.28	8.82
<i>Monizia spp.</i>	16.52	24.26
<i>Muellerius capillaris</i>	33.48	4.41
<i>Trichostrongylus spp.</i>	51.58	34.55
<i>Haemonchus contortus</i>	25.89	15.44
<i>Cooperia spp.</i>	5.35	2.94
<i>Oesophagostomum spp.</i>	4.01	1.47
<i>Trichuris spp.</i>	8.03	3.67
<i>Nematodirus spp.</i>	0	2.94

On the other hand, male sheep were found to be more often infected than females (Fig.4). However, the sex related incidence was not significant. The most prevalent gastrointestinal nematode in both species of hosts was *Trichostrongylus sp.* (Table-1). The species diversity of various parasites found in goat and sheep is given in table-1 which shows that some species can be considered as common to both species of host.

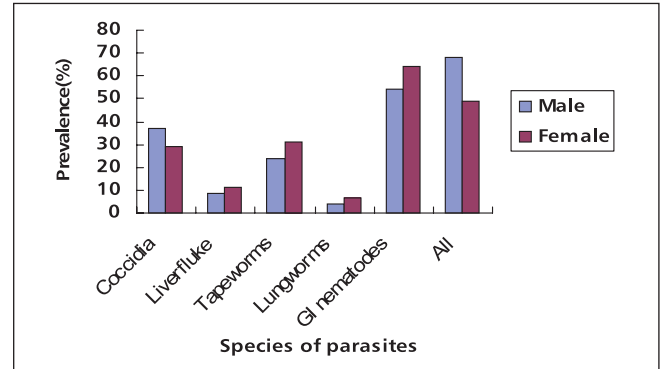


Figure 4: Extensiveness of parasitic infections in males and females of sheep

Discussion

The results of the present study reveals that goats acquire a lower level of immunity to gastrointestinal parasites than sheep and those parasitic infections are more prevalent in goats than in sheep in Bangladesh. This may be due to the gastrointestinal physiology of sheep. Bengal sheep may be genetically more resistant to GI parasites than goats.

About 48% of goats and 31% of sheep were infected with *Eimeria sp.* (coccidia). *Eimeria* infections can result in serious clinical signs of fluid diarrhea, which may or may not contain mucous or blood, dehydration, emaciation, weakness, loss of appetite, and death. Some goats may instead be constipated and suddenly die without diarrhea (9). The rate of incidence of this pathogenic protozoan species in

small ruminants in Bangladesh is awfully high though they couldn't be compared due to unavailability of available information regarding this. However, it has been found as one of the most devastating protozoa for sheep and goat farming.

Fasciola gigantica is the only liver fluke found in Bangladesh (10). In this study, the incidence of this species was 14.28% and 8.82% in goat and sheep respectively. Bhuyan (11) reported the incidence of liver fluke in goat as 13%. No available information was found for sheep in this regard. Although goat showed more incidences of almost all parasites, interestingly the incidence of tape worms was higher in sheep (24.26%) than in goat (16.52%). The reason(s) of such high prevalence in sheep is not clear, further study may be of value to conclude it precisely.

One of the most prevalent infections in goats in some part of Bangladesh seems to be *Muellerius capillaries* (Table-1; figure-3), which was found 8 times more infective to goat. Goat may be genetically more vulnerable to lungworm infection. The development of *Muellerius spp.* in the lung of goats is associated with marked tissue damage and pronounced cellular reaction (12). Infections due to *Muellerius capillaris* tend to be cumulative over time, i.e. older goats are more likely to exhibit clinical signs of coughing and ill-thrift due to heavy infestations than are young goats (12). *Muellerius* infection is more likely to be pathogenic in goats than in sheep. Normal treatment with anthelmintics may fail to clear infections, although repeat dosing and/or increasing the dose rate may clear some mild to moderate infections.

Out of Trichostrongylidae family the highest prevalence of infections showed nematodes of genus *Trichostrongylus*. Most of the species of the genus of *Trichostrongylus* actually live in the small intestine. Only one *T. axei* lives in the abomasum. The intestinal species cause more problems than *T. axei* which lives in the abomasums. The presence of the intestinal species cause diarrhea, weight loss and loss of appetite. The worms suck blood from the lining of the intestines which causes irritation and swelling of the intestinal membrane. The damaged mucosa results in malabsorption, impaired digestion and protein loss. Heavy infestations may prove to be fatal to the young animal (13).

The second most prevalent trichostrongylid nematode both in sheep and goats was a blood sucking nematode, *Haemonchus contortus*. Severe infestations can cause death of the animal within a week of heavy infection without showing any clinical signs. Animals with chronic infections show anemia and

weight loss. The worms tend to infect mostly young animals, however, older animals can develop heavy infections especially during lactation which may prove fatal. In the late stages of infections, the animal develops a swelling beneath the lower jaw, called bottle jaw (13).

The reason for absence of *Nematodirus* species in goat in this study is not clear. Although *Ostertegia*, *cooperia* and *nematodirus* were reported as most prevalent genera found from pasture (14), present study findings support previous two genera but not last one for goat.

Conclusion

This study showed high incidences of GI parasites (helminth and protozoan) in goat and sheep in Bangladesh. It was found that sheep and goat share some species of GI parasites even though the incidences vary significantly. It was also noted that sheep acquires more resistance to common GI parasites than goat does. We do believe that this study will help the researchers as well as the epidemiologist to take the necessary steps regarding the control and management of GI parasitic diseases of small ruminants in Bangladesh since there is no/little concise report on this issue. Although this study is not sufficient and further extensive study should be carried out through out the country, this study might serve as a baseline for the followers.

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ZAJEDAVSKE INVAZIJE S PLOSKIMI ČRVI IN PRAŽIVALMI PRI NOMADSKI DROBNICI V BANGLADEŠU

K. B. M. S. Islam in M. J. F. A. Taimur

Povzetek: Da bi primerjali raznolikost vrst in ugotavljali prevalenco zajedavskih invazij, smo v enem letu proučili 136 ovc in 224 koz iz Bangladeša. V prebavilih in dihalih smo iskali praživali, metljaje, trakulje in valjaste črve. Pogostnost invadiranosti z notranjimi zajedavci je bila pri kozah večja (74,55 %) kot pri ovcah (55,88 %). Veliki metljaj (*F. gigantica*) je bil pogosteje najden pri kozah (14,28 %) kot pri ovcah (8,82 %), trakulje pa nasprotno bolj pogosto pri ovcah (24,26 %) kot pri kozah (16,52 %). Koze so imele tudi več praživali (*Eimeria* spp.) in pljučnih zajedavcev (*Muellerius* spp.). Pri kozah se je *Muellerius capillaris* pojavil kar v 33,48 % primerov, pri ovcah pa samo v 4,41 %. Pljučni zajedavci so bili pogostejši pri samicah, ostali zajedavci pa pri samcih. Pri obeh vrstah drobnice je bil najpogosteje ugotovljen želodčno-črevesni zajedavec *Trichostrongylus* spp., takoj za njim pa *Haemonchus*. Ugotovili smo 10 različnih vrst notranjih zajedavcev in sicer se jih je 9 pojavljalo pri obeh vrstah gostiteljev. Najpogosteje smo našli vrste *Eimeria*, *Trichostrongylus*, *Haemonchus*, *Monizia* in *Fasciola*.

Ključne besede: helminti; praživali; zajedavci; prevalenca; drobnica

RETROSPECTIVE STUDY OF CANINE PARVOVIROSIS IN SLOVENIA

Mitja Gombač^{1*}, Tanja Švara¹, Marko Tadić², Milan Pogačnik¹

¹ Institute of Pathology, Forensic and Administrative Veterinary Medicine, Veterinary Faculty, Gerbiceva 60, 1000 Ljubljana, Slovenija;

² Veterinary Faculty, Heinzelova 55, 10000 Zagreb, Croatia

*Corresponding author, E-mail: mitja.gombac@vf.uni-lj.si

Summary: The aim of this retrospective study, based on the data from the archive of the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana, was to estimate the correlation between epidemiological parameters such as gender, age and breed of dogs that died of canine parvovirus, and the months of a year in which they died.

84 dogs, aged 1 month to 4 years, of both genders and 27 different breeds, which died of canine parvovirus from January 1987 to January 2005, were included in the study. Results of this descriptive study suggest that the age of dogs and the time of year when death occurred were correlated with canine parvovirus while there was no correlation between gender or breed and incidence of death due to canine parvovirus.

Key words: veterinary medicine; pathology; canine parvovirus; parvoviral enteritis; dogs

Introduction

Canine parvovirus is a contagious viral disease of dogs. First cases of canine parvovirus had been almost simultaneously detected for the first time in the USA, Australia and Europe in 1978 (1, 2, 3). In the following three years the disease had spread to all continents (4, 5, 6). The first case of canine parvovirus in Slovenia was diagnosed in July 1980 (7).

The disease is caused by a virus belonging to the group of parvoviruses, which also includes feline panleukopenia virus (FPV; 6). It is presumed that outbreak of parvovirus in dogs was a consequence of FPV mutation (6). Canine parvovirus is caused by two dog specific parvoviruses – canine parvovirus type 1 (CPV1) and canine parvovirus type 2 (CPV2). In contrast to low pathogenicity of CPV1, which rarely causes canine parvovirus, CPV2 is highly pathogenic (6, 8, 9). Several species of wild canids, e.g. coyotes, raccoons, foxes and wolves, are also susceptible to canine parvovirus infection (6). The most typical form of the canine parvovirus is exhibited in the form of enteritis, but the disease

can also manifest as parvoviral myocarditis or as a mixed form (6). All three forms have often fatal outcome (6). Young dogs from six weeks to one year of age are the most susceptible group for canine parvovirus, afterwards the morbidity decreases. Puppies younger than six weeks are protected by maternal immunity (10).

Materials in methods

The necropsy reports from the archive of the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana were reviewed in order to find out causes of death in dogs dissected in the last 18 years, from January 1987 to January 2005. Eighty four dogs in which parvovirus was diagnosed by histopathological analysis were included in this study. In 18 dogs, parvovirus was also confirmed by immunocytochemistry.

Immunohistochemical staining

Polyclonal rabbit antibody against canine parvovirus was used for immunolabelling. Serum was

raised in rabbits which were immunised with the suspension of canine parvovirus, isolat LJ-80, which was replicated on the cell culture of cell line FER (Feline Embryonal Kidney), ECACC, No 90031401. Hemagglutination titer of viral suspension was 1: 1024 (11).

Four micrometers thick, deparafinised tissue sections of jejunum, ileum, liver, kidney, spleen, thymus, lymph nodes and lung were incubated in citrate buffer (pH= 6.0) in the microwave oven for 15 minutes to expose the antigens, cooled to the room temperature and incubated in 3% hydrogen peroxide for 1 hour to inhibit the endogenous peroxidase. Polyclonal rabbit antibodies against canine parvovirus, were diluted 1: 400 in 5% bovine serum albumin in phosphate buffer saline (PBS). Tissue sections were incubated with primary antibodies at room temperature for 30 minutes, followed by washing in PBS for 5 minutes, incubated with biotinylated mixture of goat anti-rabbit and goat anti-mouse secondary antibodies (LSAB[®]2 HRP visualization system, DAKO) for 20 minutes, rewashed with PBS, incubated in streptavidin conjugated with peroxidase (DAKO) and finally incubated in 1% 3-diaminobenzidine tetrahydrochloride, containing 0.03% hydrogen peroxide, for 7 minutes. Sections were counterstained with haematoxylin, dehydrated and mounted with resin (11).

Statistical analyses

Data about gender, age and breed of dogs and month of their death was determined from the records about anamnesis.

For determination of effects of chosen epidemiological parameters on canine parvovirus, dogs were divided into two groups by gender: 70 males and 14 females; in three groups by age: 50 puppies up to 6 months of age, 19 dogs from 6 months to 1 year and 5 dogs older than 1 year; into four groups by breed: 14 German Shepherds, 9 Rottweilers, 6 Labrador Retrievers and 20 mixed breeds.

Regarding the month of the death, dogs were divided into 12 groups, and by the year of death into 18 groups.

Relative ratio of deaths caused by parvovirus from the total number of dissected dogs (2486) was calculated individually for each of the above groups. The differences between groups for each epidemiological parameter were determined using standard statistical methods: correlation and regression analysis, t-test and chi test, with $p < 0.05$, which were considered as statistically significant.

Results

Anamnesis and diagnosis of canine parvovirus

In the last 18 years, 2486 dog carcasses underwent pathological examination at the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana in the last 18 years. Typical macroscopic lesions in the small intestine (mostly hemorrhagic enteritis), which were diagnosed as parvoviral enteritis using histopathological examination, were found in 84 dogs (3.4 % of all examined dogs).

In 55 dogs that died due to parvovirus infection, sudden onset with vomiting, bloody diarrhea, depression and loss of appetite were noticed by their owners or veterinarians. All these dogs died in one or at most two days after the onset, or were euthanized due to severe illness. Five dogs died suddenly, without previous clinical signs. For 24 dogs there was no anamnesis concerning the clinical status prior to death.

Ten dogs were vaccinated against canine parvovirus, three dogs were not vaccinated while for 71 dogs there were no data concerning the vaccination.

Epidemiological analysis

Sex of dogs

Seventy dogs (83.3 %) that died due to canine parvovirus were males and 14 (16.7 %) were females (Diagram 1). Statistical analysis revealed significant difference between sexes in dogs that died due to parvovirus ($P < 0.01$).

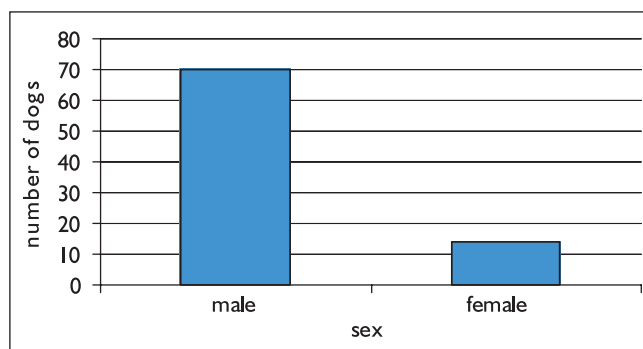


Diagram 1: Sex ratio of dogs that died due to canine parvovirus

The age of dogs

Fifty dogs (67.6 %) that died of canine parvovirus were younger than six months, 19 dogs (25.7 %)

were aged from six months to one year, and only five dogs (6.8 %) were older than one year. For 10 dogs included in our study there were no data about their age (Diagram 2). Comparison between age groups and the death due to canine parvovirus revealed statistical significance between groups ($P < 0.01$).

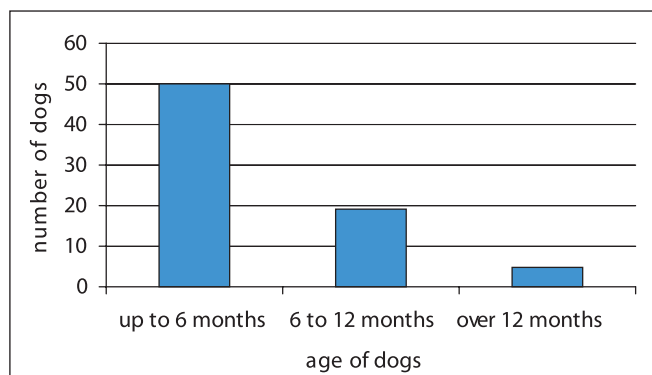


Diagram 2: Age distribution of dogs that died due to canine parvovirus

Breeds of dogs

The highest percentage of deaths, 23.8 % (20 dogs) was noticed in mixed breeds, followed by German Shepherds with 16.7 % (14 dogs), Rottweilers with 10.5 % (nine dogs), Labrador Retrievers with 7.1 % (six dogs) and Poodles with 5.9 % (five dogs). Single cases of canine parvovirus were diagnosed in other 21 pure breeds. The differences between different breeds and the number of deaths due canine parvovirus was not statistically significant ($P > 0.05$).

The year of the death

Twenty dogs died due to canine parvovirus in 1991 (10.3 % of all dogs). In 1987 and from 2001 to 2003 there were no deaths due to canine parvovirus. The percentage of deaths in other years ranged from 1.29 to 8.4 %.

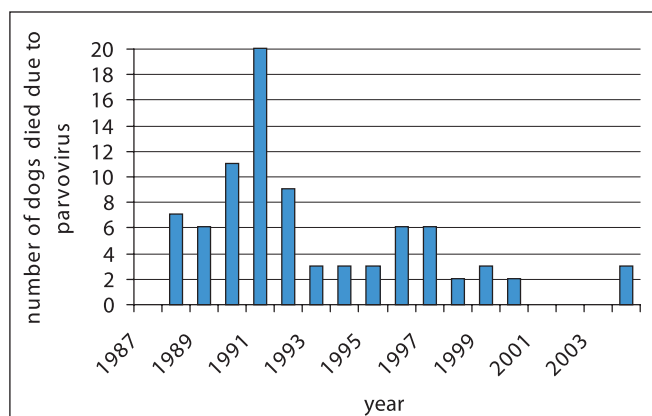


Diagram 3: Number of dogs that died due to canine parvovirus in the last 18 years

The month of the death

The highest percentage of deaths due to canine parvovirus was noticed in August (16.7 %), followed by September (11.9 %), October, December and January (10.7 % each), May (9.5 %) and November (7.1%). The percentage of deaths ranged from 2.3 to 5.9 % in other months of the year (Diagram 4). The difference between months and deaths due to canine parvovirus was statistically significant ($P < 0.001$).

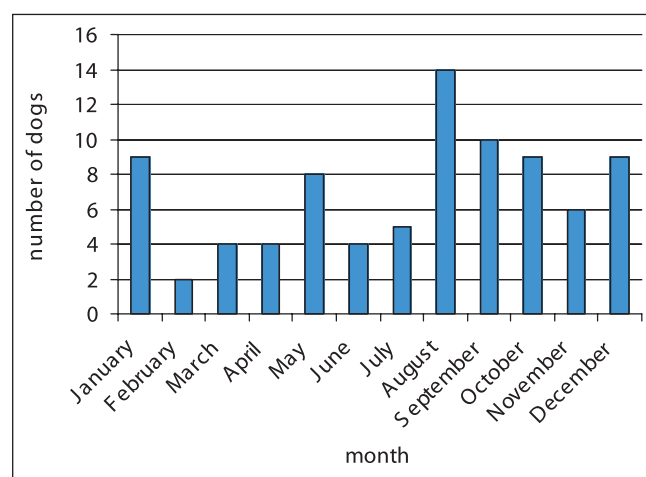


Diagram 4: Number of dogs that died due to canine parvovirus by the month in which they died.

Discussion

In the last 18 years, 2486 dogs were dissected at the Institute of Pathology, Forensic and Administrative Veterinary Medicine of Veterinary Faculty in Ljubljana and among them, 84 died or were euthanized due to canine parvovirus. In all cases, canine parvovirus was suspected due to clinical signs and/or characteristic intestinal lesions, and was confirmed by histopathological examination of the intestine and in 18 dogs also with immunohistochemical reaction using polyclonal antibodies against canine parvovirus (11). Several authors described microscopic changes in small intestine, i.e. necrosis of crypt's epithelia with crypt's dilatation, atrophy of intestinal villi, necrotic enterocytes with enlarged nucleus and focal mucosal collapse, as typical for parvovirus (6, 7, 18). Similar lesions were found also in the small intestines of dogs included in our retrospective study. Ruiz Romero et al. (19) also found necrosis of crypts and atrophy of villi in small intestine of dogs and confirmed parvovirus by immunohistochemical staining (19).

Since 1980, when the first case of canine parvovirus has been diagnosed and described in Slov-

enia (7), deaths due to canine parvovirus were diagnosed almost every year. The number of deaths increased in 1990, 1991 and in 1992, while a slow fall was noticed afterwards until 2001, when no case of death due to canine parvovirus was diagnosed. The first new outbreak occurred in November 2004. There is no data in the literature concerning the disease frequency in the world during the last decades.

The most likely reason for decreased number of deaths due to canine parvovirus is greater owner's awareness and regular preventive vaccination against canine parvovirus. The new outbreak of canine parvovirus could be related to the new canine parvovirus mutant that has been just found in Italy (12). This hypothesis is supported by the facts that this was the first outbreak of disease after three years and, that all three puppies were vaccinated with standard vaccine against canine parvovirus. The second possible explanation for the new outbreak is inappropriate time of vaccination. It is known that there is a critical period from two to four weeks, when vaccination is fairly unsuccessful. In this period, the maternal antibody titer is so high, that a puppy is unsusceptible to preventive vaccination, but at the same time too low to protect the animal against infection. For that reason the vaccination in the above cited period is ineffective. Therefore it is suggested that the assessment of the titer of antibodies against canine parvovirus should be determined before preventive vaccination (13).

83.3 % of dogs, which died due to canine parvovirus, were males, and 16.7 % were females. The difference between genders in the death ratio due to canine parvovirus is statistically significant. In spite of this result we cannot conclude that males are more susceptible for canine parvovirus infection. The major susceptibility of male dogs will be confirmed, if there will be an equal number of male and female dogs in the Slovenian dogs' population. Unfortunately, we don't have the data about the exact number of male and female dogs in Slovenia. Other authors (4) reported that the canine parvovirus infection was more frequent in male dogs, but statistical analyses could not confirm significant differences between sexes and morbidity (4, 5).

The highest percentage of deaths, 67.7 %, was noticed in dogs younger than six months, and the lowest, 6.7 %, in dogs older than one year. From six months to one year 25.7 % of dogs died due to canine parvovirus. Comparison between the age groups and death due to canine parvovirus showed statis-

tical significance. We have demonstrated that age affects the death due to canine parvovirus. Very high percentage of deaths, 80 %, was found in puppies younger than six months by Pospischill and Yamaho (14) and 74.5 % morbidity was reported by Ernst et al. (4) at the same age. The most likely reason that dogs younger than six months are at the greatest risk is very high affinity of canine parvovirus for tissues with high mitotic activity (10). Puppy's immune system, which depends on the maternal antibodies, also has an important role in the fight against canine parvovirus (15). Maternal immunity which lasts two to three months makes morbidity of puppies at this age very low. Singular cases of canine parvovirus in puppies are probably a consequence of subclinical form of canine parvovirus during the mother's pregnancy (4). The mitotic activity in the cells decreases with age, therefore the percentage of older dogs that die due to canine parvovirus is very low. Deaths in older dogs are mostly related to the high invasions with intestinal parasites, bacterial and viral infections that all cause immunosuppression, damage of intestinal mucosa and consequently provoke increased mitotic activity of intestinal cells (4). The statistically significant difference between the age of dogs and morbidity due to canine parvovirus was reported in the study of Ernst et al. (4).

23.8 % of Slovenian dogs, which died due to canine parvovirus, were mixed breeds, followed by German Shepherds, Rottweilers, Labrador Retrievers and Poodles. Using statistical analysis we found that the difference between groups of different breeds and the number of deaths due to canine parvovirus was not statistically significant. We have demonstrated that breed does not have influence on death due to canine parvovirus. Several authors wrote about greater susceptibility of some pure breeds, i. e. Setters (4), Pointers (4), Doberman Pinschers (4, 16, 17) and Rottweilers (4, 16). Pospischill and Yamaho (14) and Houston et al. (17) established that German Shepherds are the most susceptible breed for canine parvovirus. According to statistical data from the year 1996, we noticed that German Shepherds and Rottweilers, followed by Labrador Retrievers and Poodles were the most numerous amongst pure breed's offspring in Slovenian dog's population. In our study we found out that these are breeds with the highest percentage of death due to canine parvovirus.

Most of Slovenian dogs died due to canine parvovirus from August to January. The highest percentage of deaths was reported in August, which is one

of the warmest months in Slovenia, followed by September. High daily temperatures induce loss of appetite, decrease function of the immune system and increase susceptibility to bacterial, parasitic and viral diseases. In the literature we found out that the highest titer of the antibodies against canine parvovirus was determined during summer months (4). Pospischill and Yamaho (14) reported the highest incidence of canine parvovirus in November, December and January and the lowest number in June, July and September. Increased number of deaths during winter could be related to low temperatures which also suppress immune system. Using statistical analysis we demonstrated that the difference between months and the number of deaths due to canine parvovirus was statistically significant. We can conclude that very warm and very cold months have influence on the death due to canine parvovirus. Ernst et al. (4, 5) also demonstrated that the difference between months and morbidity due to canine parvovirus is statistically significant.

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RETROSPEKTIVNA ŠTUDIJA PARVOVIROZE PRI PSIH V SLOVENIJI

M. Gombač, Tanja Švara, M. Tadić, M. Pogačnik

Povzetek: Retrospektivno študijo parvoviroze pri slovenskih psih smo opravili z namenom, da bi ugotovili morebitno povezavo med spolom, starostjo, pasmo psov in mesecem, v katerem so psi poginili, ter parvovirozo. Anamnestične podatke o raztelesenih psih, poginulih od januarja 1987 do januarja 2005, ter podatke o vzrokih njihovega poginov ali usmrtilcev smo vzeli iz arhiva Inštituta za patologijo, sodno in upravno veterinarstvo Veterinarske fakultete v Ljubljani. Parvoviroza je bila diagnosticirana s patohistološko preiskavo črevesja, v katerem parvovirusi povzročajo za to bolezen patognomonične spremembe, pri 18 psih pa smo bolezen potrdili tudi z imunohistokemično preiskavo črevesja in nekaterih parenhimskih organov.

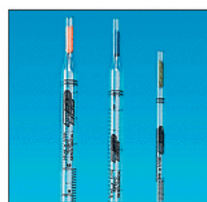
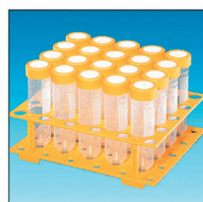
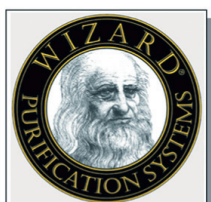
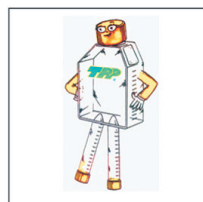
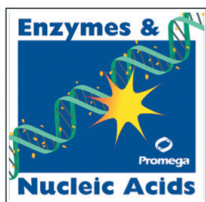
Od januarja 1987 do januarja 2005 je bila na Inštitutu opravljena raztelesba 2486 psov, od katerih jih je 84 (3,4 %) poginilo zaradi parvoviroze. Največ poginov zaradi parvoviroze je bilo zabeleženih v avgustu (16,7 %) in septembru (11,9 %), v oktobru, decembru in januarju pa je bilo poginov nekoliko manj (10,7 %). Najmanj psov je zaradi parvoviroze poginilo v februarju. 67,6 % psov je bilo ob poginu starih do 6 mesecev, le 6,7 % psov pa je bilo starejših od enega leta. 83,3 % psov je bilo moškega spola, med čistokrvnimi psi pa je bila parvoviroza najpogosteje diagnosticirana pri nemških ovčarjih, rotvajlerjih in labradorcih.

Na osnovi statističnih analiz podatkov smo ugotovili, da so starost psov in meseci v letu v povezavi s poginom zaradi parvoviroze, spol in pasma pa ne.

Ključne besede: veterinarska medicina, patologija; parvoviroza; parvovirusni enteritis, psi

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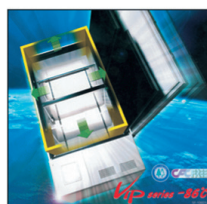
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IZDELKI ZA MOLEKULARNO BIOLOGIJO

**DOKUMENTACIJA
IN ANALIZA GELOV**

PLASTIKA ZA CELIČNE KULTURE



ČISTA VODA ZA LABORATORIJ

**SKRINJE
IN HLADILNIKI**

**CELIČNE KULTURE, GELI
IN MOLEKULARNA BIOLOGIJA**



ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

**DIAGNOSTIKA
MIKOPLAZEM
IN LEGIONEL**

**HPLC in GC
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Article in proceedings of a meeting or symposium: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

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