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# SLOVENIAN VETERINARY RESEARCH

## SLOVENSKI VETERINARSKI ZBORNIK


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**Original Research Paper**

# LONGEVITY OF LASTING SPECIFIC IMMUNITY AFTER PRIMARY VACCINATION AGAINST RABIES – COMPARISON OF ELISA AND FAVN TESTS

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**Summary:** The purpose of the study was to monitor the efficacy of primary vaccination against rabies and the need for booster doses. These studies validate at the same time the recent technological improvements in laboratory diagnostics of the level of rabies protection in human sera. Study was done at the level of antibodies considering that an antibody titer ≥ 0.5 IU/ml is protective. We used Platelia rabies ELISA kit (BIO-RAD Laboratories) for the detection of rabies anti-glycoprotein antibodies in 41 human sera of previously healthy veterinarian students. Neutralising rabies antibodies were measured simultaneously by fluorescent antibody virus neutralization (FAVN) test as well. Subjects entering the study have received 2 to 8 years prior rabies treatment with human diploid cell vaccine (HDCV, Rabivac, Chiron Germany) according to schedule: one vaccine on 1, 7, 21 and 365 day. Mean level of rabies antibody detected by ELISA was 19.6 EU/ml (SD 18.8 minimum 1 maximum 56). Results were higher in the groups vaccinated recently. Nobody had titer ≤ 0.5 IU/ml either in ELISA or in FAVN test. In the FAVN test, the average titers were higher and reached 54.4 IU/ml (SD 44.3 minimum 0.7 maximum 152.5). An immune-complex-like reaction occurring after administration of the booster doses of rabies vaccine is one of the reasons to reconsideration of the needs for administration of booster rabies vaccines. At the same time, the need for mass protection of subjects exposed to rabies virus professionally is existing worldwide . The results of these studies indicate that HDCV is highly immunogenic in both FAVN test and ELISA tests. High level of protection is lasting in human sera for at least 8 years. Average levels of detected rabies antibodies were lower in ELISA in comparison with FAVN test. Correlation between two tests was found.

**Key words:** rabies; rabies vaccines – drug effects – pharmacology; drug evaluation, serodiagnosis – methods; antibodies, viral – analysis; enzyme-linked immunoassay; fluorescent antibody technique; comparative study

## Introduction

Published data on the longevity of lasting specific immunity after primary vaccination against rabies have approved the fact that neutralising antibody level after vaccination is protective for at least two upcoming years (1). According to the recommendation of World Health Organization and Centre for Disease Control, sufficient level of rabies neutralising antibody appointed at 0.5 IU/ml (2, 3). For persons continuously professionally exposed to rabies virus, it is recommended to have a serum sample tested for rabies antibodies between 6 months and two years. Intervals for testing depend on the level of exposure. In Slovenia, rabies researchers need to be laboratory tested every 12 months, employees for rabies diagnostics laboratory once a year, veterinarians every two years. If the titers proved by the rapid fluorescent focus inhibition test (RFFIT) are below 0.5 IU/ml, booster vaccination with one vaccination dose is indicated (2). Pre-exposure immunization for rabies is necessary for spelunkers, bat researchers, animal control and wildlife workers in rabies epiz-

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ootic areas, veterinary students as well as travelers visiting areas where rabies is enzootic and where immediate access to appropriate medical care, including biologicals, is limited (4).

Several serological tests for the detection of rabies virus neutralization antibodies have been described. The first test was a mouse neutralization test (MNT) developed by Webster et al. (5). The most commonly used technique for detection of protective level of rabies antibodies in sera of animals and humans is the rapid fluorescent focus inhibition test (RFFIT) developed in 1973 (6). Another cell culture based technique – the fluorescent antibody virus neutralization (FAVN) test has been shown to be more specific than the RFFIT test (7). The FAVN test is based on neutralization of rabies virus using cell culture. The reading and interpretation of results are less subjective than RFFIT, because use an "all or nothing" method of reading. Several indirect ELISA tests (Enzyme-Linked Immunosorbent Assay) that incorporate rabies glycoprotein/anti-human immunoglobulin/enzyme conjugates have been described for human (8) and animal post vaccination rabies antibodies titrations (9). The Platelia Rage kit incorporates protein A but has rabies virus glycoprotein as the coating antigen.

The aim of our study was to detect level and duration of rabies antibody in the sera of pre-exposure treated persons and to compare two laboratory tests for that purposes – FAVN and ELISA tests.

## Material and methods

The immunogenity of a human diploid cell vaccine (HDCV) was evaluated using veterinary medical students. 41 healthy adults were enrolled in our trial. A person was excluded from enrolment if he/she had a previous history of additional rabies vaccination, had a history of any immunosuppresive disease or chronic disorders, oral or parenteral immunosuppressive therapy.

Mean age of subjects at the time of entering the study (year 2004) was 25.3 (SD 2.6 median 25 minimum 22 maximum 33). At the beginning of the vaccination, subjects were between 20 and 29 years (mean 21.1 SD 1.4) old. We have tested 12 men and 29 female.

Four 1.0 ml injections of human diploid cell vaccines HDCV (Rabivac, Chiron Vaccines, Germany) were administered intramuscularly in deltoid area on days 1, 8, 22 and 365 in the course of pre-exposure treatment: 16 persons have received this pre-exposure treatment against rabies 2 years before blood sampling, 9 persons 4 years before, 4 persons 5 years before, 5 persons 6 years before and 7 persons 8 years before blood sampling. The Human Research Board at Ministry of Health of Republic Slovenia approved the study protocols and informed consent forms signed by all subjects.

Blood samples were collected from subjects and sent to laboratory for testing rabies virus antibodies after one to two months after last administration of vaccine. After the collection of blood sample it was centrifuged for 10 min at 1.000 g, aliquoted and stored at –20 +/- 2°C. Rabies virus neutralizing antibody levels were measured in human sera using the Fluorescent Antibody Virus Neutralization (FAVN) (7) in Department of Virology at Veterinary Faculty of University of Ljubljana. All sera were heat inactivated (for 30 min at 56°C) and analysed for the presence of rabies virus antibodies with fluorescent antibody virus neutralisation (FAVN) test. The method was described by Cliquet and co-workers (7). Briefly, the serial three-fold dilutions of serum samples were prepared in duplicate in minimum essential medium (MEM, Gibco, Paisley, UK) and were placed on a 96-well microplate. A challenge virus strain (CVS, obtained from OIE/WHO, Nuncy, France) in titre 30-200 TCID50/0.1 ml was added in each well. After incubation for 60 min at 37°C BHK cells were added. The cells were fixed after 48 hours of incubation with cold acetone (stored at –20°C) and stained with anti-rabies fluorescent conjugate (Sanofi Diagnostic Pasteur, Marnes-la-Coquette, France). The highest neutralising antibody titre of the serum samples was considered the dilution, which completely blocked CVS propagation. The OIE/WHO reference positive serum with known neutralising antibody titre (0.5 IU/ml), negative serum controls, virus and cell controls were also included in the tests. The neutralising antibody titres obtained in sera were transformed into International Units/ml (IU/ml). Geometric mean titre (GMT) was determined by software Excel.

The same samples were tested in ELISA test in Blood Transfusion Center of Slovenia. The test assay was the PLATELIA ELISA (BIO-RAD, Marnes-la-Coquette, France). PLATELIA rabies kit is an immunoenzymatic technique for the detection of rabies virus anti-glycoprotein antibodies in human serum and plasma.

Subjects were considered protected against rabies virus infection if they achieved a FAVN test or ELISA titers of ≥ 0.5 IU/ml. FAVN test, measuring

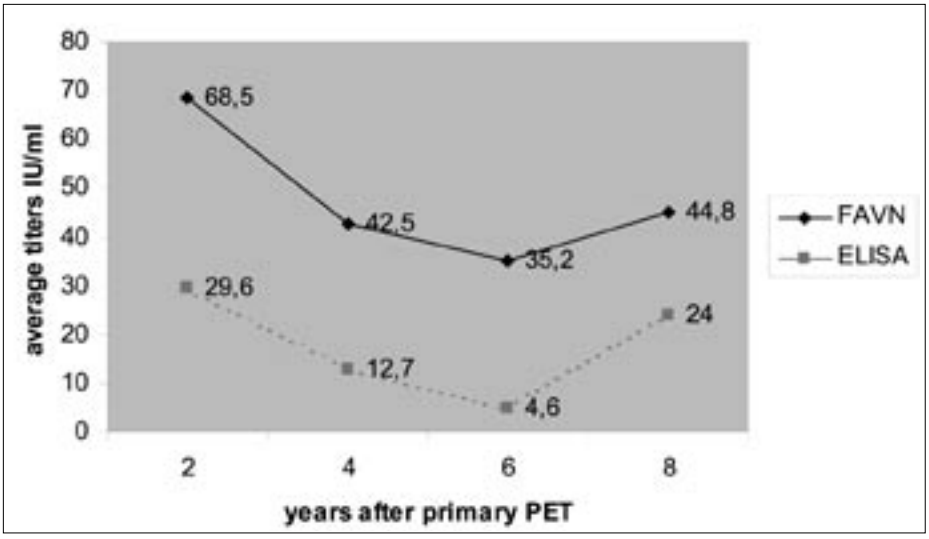


**Figure 1:** Average level of rabies antibodies in ELISA and FAVN tests – years after the primary pre-exposure vaccination

neutralizing antibodies, was used as the reference test.

All statistical analyses were done before the code was broken. Statistical analyses were performed using the SPSS System for Windows version.

## Results

100 % of the subjects in each tested groups had post-vaccination rabies antibodies titers > 0.5 IU/ml in both FAVN and ELISA tested methods. For surveillance of exposed subjects, WHO (2) considers that high levels of rabies antibody in RFFIT protect subjects exposed to risks of rabies. Results of these studies indicate that HDCV administered intramuscularly to healthy adults previously vaccinated against rabies 2 to 8 years before the study and according to schedule 1, 7, 21 and 365 is excellently immunogenic for both glycoprotein and neutralizing antibodies synthesis. High levels of antibodies were detected in both tests (see table 1). Correlation between values of titers measured in ELISA and FAVN tests using Spearman's Correlation Coefficient was significant at the 0.01 level (2-tailed), indicating correspondence of results in both tests.

Absolute values in each group (FAVN and ELISA) were more or less different. Table 1 and Figure 1 show that average means of antibodies levels were usually higher in the groups vaccinated recently and with the FAVN test. Comparison in the average level of antibodies in years after primary pre-exposure vaccination revealed that FAVN and ELISA tests were equally able to detect decrease of level of antibodies with years after the start of vaccination. Increasing levels of rabies antibodies in both tests 8 years after primary vaccination were observed in persons who were eventually already professionally exposed as veterinarians to rabies virus.

HDCV vaccine met the immunogenicity goal of producing rabies virus neutralization and glycoprotein antibodies titers. Comparative study showed that all subjects had neutralizing as well as anti-glycoprotein antibodies levels well above the satisfying level even 8 years after the start of the pre-exposure treatment.

## Discusion

We suppose that different kinds of rabies vaccines and different vaccination schedules have influence on the achieved level and the duration of protective antibody levels against rabies. The laboratory assays used for rabies antibody titration and type of rabies antibodies are important as well. Different laboratory assays are referenced for clinical decisions of many infectious diseases. Interpretation of them is sometimes a difficult task. In our study, rabies antibody response were assessed by two laboratory assays (ELISA and FAVN test) High titers were obtained with both test therefore there is any concern about protective levels of rabies pre-exposure treatment.

In our study we have commonly detected much higher protective level of anti-rabies antibodies as officially recognized as lower protective level by WHO (2) and Center for Diseases Control Atlanta (3). Tokoyama and his coworkers published similar observations several years ago (10).

**Table 1:** Results of rabies neutralising antibodies in 41 persons according to FAVN test and ELISA

| Individuals | Number of years between the first vaccinations and blood sampling | Test FAVN | | Test ELISA | |
|---|---|---|---|---|---|
| | | (IU/ml) | Mean per group IU/ml) | (EU/ml) | Mean per group IU/ml) |
| 1 | 2 | 15,5 | | 6 | |
| 2 | 2 | 33,5 | | 7 | |
| 3 | 2 | 19,4 | | 3 | |
| 4 | 2 | 48,5 | | 17 | |
| 5 | 2 | 41,3 | | 12 | |
| 6 | 2 | 52,5 | | 34 | |
| 7 | 2 | 79,4 | | 56 | |
| 8 | 2 | 113,7 | | 6 | |
| 9 | 2 | 43,1 | | 65 | |
| 10 | 2 | 64,1 | | 9 | |
| 11 | 2 | 113,7 | | 15 | |
| 12 | 2 | 152,0 | | 18 | |
| 13 | 2 | 111,5 | | 52 | |
| 14 | 2 | 105,6 | | 13 | |
| 15 | 2 | 121,4 | | 2 | |
| 16 | 2 | 1,8 | 69,8 | 19 | 20,8 |
| 17 | 4 | 27,1 | | 4 | |
| 18 | 4 | 123,2 | | 52 | |
| 19 | 4 | 132,8 | | 7 | |
| 20 | 4 | 5,4 | | 30 | |
| 21 | 4 | 110,5 | | 8 | |
| 22 | 4 | 115,6 | | 44 | |
| 23 | 4 | 7,5 | | 2 | |
| 24 | 4 | 42,4 | | 15 | |
| 25 | 4 | 48,2 | 68,0 | 9 | 19,0 |
| 26 | 5 | 27,2 | | 19 | |
| 27 | 5 | 62,1 | | 5 | |
| 28 | 5 | 2,1 | | 7 | |
| 29 | 5 | 64,1 | 38,8 | 9 | 10,0 |
| 30 | 6 | 60,8 | | 18 | |
| 31 | 6 | 42,1 | | 44 | |
| 32 | 6 | 22,3 | | 15 | |
| 33 | 6 | 46,8 | | 56 | |
| 34 | 6 | 108,5 | 56,1 | 56 | 37,8 |
| 35 | 8 | 4,15 | | 5 | |
| 36 | 8 | 30,0 | | 52 | |
| 37 | 8 | 42,3 | | 16 | |
| 38 | 8 | 15,6 | | 8 | |
| 39 | 8 | 13,0 | | 7 | |
| 40 | 8 | 7,1 | | 5 | |
| 41 | 8 | 15,9 | 18,3 | 8 | 14,4 |

Pre-exposure vaccination against rabies is usually performed with HDCV, a human diploid cell vaccine PCEC, a purified chick embryo cell vaccine; a RVA, rabies vaccine adsorbed (2). One year apart from the start of the treatment four dose HDCV rabies vaccine schedule used in our study, is recommended by vaccine producer of vaccine and some authors (11, 12, 13) as one of possible schedule for pre-exposure treatment. Existence of individual immune reactions following rabies vaccination, as for other vaccina-

tions is obvious and non-responders could be expected after rabies vaccination as well. Therefore clinical decisions should be made individually from case to case. Average level of protection is interesting more for research purposes. After accidental exposition of vaccines to the rabies virus, an additional dose of rabies vaccine is highly recommended in spite of probably high level of neutralizing antibodies.

Further studies are necessary to give evidence if high level of achieved protection with HDCV and with presented vaccination pre-exposure schedules is connected with even more than 8 years lasting immunity.

We suppose that fourth dose in pre-exposure schedule is definitely not obligated, but if a fourth dose is given as booster after one year from the start of vaccination, substantially prolongation of the protection could be expected. People at continued risk for rabies exposure should consider acceptance of the presented regimen and regular boosting with rabies vaccine as well.

Serological testing may be the useful way for reducing the number of rabies vaccine doses in the course of booster. Enhanced surveillance of the necessity for the start of pre-exposure protection and booster is advisable. In this way, the total number of professionally exposed persons to treat, who regularly need boosters, could be reduced and undesirable side effects of vaccinations as well. We predict that the use of pre-exposure rabies vaccination could in such a way even increase the demand for pre-exposure preventive treatment. In this way the total protection of professionally exposed subjects would be posed on higher level. An immune-complex-like reaction occurs after administration of the booster doses of HDCV (13). Local reactions (14) and systemic hypersensitivity reactions after booster vaccinations with HDCV (15) have been reported as well. These reactions are the additional reason so take into consideration very carefully when establishing the need for administration of booster rabies vaccines. However, rabies treatment save lives and it should be accepted as compulsory.

PLATELIA rabies kit is an immunoenzymatic technique for the detection of rabies virus antibodies in serum or plasma of human and several animal species. Some authors have used ELISA tests for detection of rabies virus antibodies in human sera (8). This way it can be used for monitoring the efficiency of vaccine testing on laboratory and field animals, and also as a research tool for monitoring the antibody titer of vaccinated subjects. The present study demonstrates that ELISA method (Platelia kit) provides very high level of detection and that correlation exists between ELISA and FAVN test results. Correlation between neutralization assay and ELISA and correlation with the FAVN test was shown in recently published study of Arai et al. (16) with higher neutralisation than ELISA titers for most samples. Results of this study are in accordance with the results revealed in our study.

Cliquet and co-workers (9) have provided another ELISA with lower sensitivity than the FAVN test. It is a useful tool for rapidly screening serum samples (retesting of ELISA negative results by a reference technique is recommended by OIE) from vaccinated companion animals and the ELISA compared favorably with data generated using the FAVN test. The major advantages of the ELISA test are that it can be completed in several hours, does not require the use of live virus and can be performed without the need for specialized laboratory containment. This is in contrasts with several days needed for conventional rabies antibody virus neutralization assays. According to the authors, ELISA assay would be a valuable screening tool for the detection of rabies antibodies in vaccinated domestic animals in combination with other prescribed serological tests. We propose that the same consideration could be accepted for human sera as well. Comparison with RFFIT or equally worthwile FAVN (17) will be very interesting.

Prevention of diseases in professionally exposed persons is one of the priorities in public health sector (18, 19). According to our opinions and opinions of some other authors (20), monitoring the titers of antibodies with consistent and validation laboratory assays could be a useful contemporary method for making decisions for the purpose to give booster or not in the professionally exposed persons to rabies. Similarly, it could be the best way to allow a decrease in the number of boosters in the cases of long lasting professional career.

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## TRAJANJE SPECIFIČNE IMUNOSTI PO PRIMARNEM CEPLJENJU PROTI STEKLINI – PRIMERJAVA TESTOV ELISA IN FAVN

M. Stantić-Pavlinić, S. Levičnik-Stezinar, L. Zaletel-Kragelj, P. Hostnik

**Povzetek:** Namen študije je bil opraviti kontrolo uspešnosti primarnega cepljenja proti steklini in dobiti odgovor na vprašanje ali je morebiti potrebno še dodatno cepljenje. Istočasno smo želeli preveriti laboratorijske izboljšave pri metodah določanja stopnje zaščitnih protiteles proti virusu stekline v serumih ljudi. Študija je izhajali iz podatka, da titer protiteles enak ali višji od 0,5 IU/ml, ščiti človeka pred steklino.V serumu 41 študentov veterine smo v testu ELISA, komplet Platelia (BIO-RAD Laboratories), določali protitelesa proti glikoproteinu virusa stekline. Hkrati smo nivo protiteles določali tudi v seronevtralizacijskem testu z imunofluorescenco (test FAVN). Študenti so bili cepljeni 2 do 8 let pred odvzemom krvnega vzorca s cepivom proti steklini (HDCV, Rabivac, Chiron Germany) po shemi: ena doza 1., 7., 21. in 365. dan. Povprečni titer protiteles, ugotovljen v testu ELISA, je znašal 19,6 EU/ml (SD 18,8 minimum 1 maximum 56). Višje titre smo ugotovili pri skupini, ki je bila cepljena pred kratkim. Nihče ni imel titra protiteles nižjega od 0,5 IU/ml. V testu FAVN smo dobili nekoliko višji titer protiteles, saj je znašal 54,4 IU/ml (SD 44,3, minimum 0,7, maksimum152,5). Nivo protiteles proti virusu stekline v serumu pacienta je uporaben kazalnik potrebe po izvedbi revakcinacije. Hkrati ugotavjamo, da je upravičena široka zaščita proti steklini tistih oseb, ki so poklicno izpostavljene večji možnosti okužbe z virusom stekline. Rezultati te študije kažejo, tako v testu ELISA kot v testu FAVN, da daje cepivo HDCV zadovoljivo zaščito. Ugotovili smo visok titer protiteles še 8 let po izvedbi osnovnega cepljenja. V testu ELISA smo v povprečju ugotovili nižji titer protiteles kot v testu FAVN.

**Ključne besede:** steklina; steklina, cepiva – učinki zdravil – farmakologija; zdravilo, ocena; serodiagnostika – metode; protitelesa, virusna – analize; ELISA; imunofluorescentna tehnika; primerjalna študija

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**Original Research Paper**

# CANINE LYMPHOMA: CYTOLOGIC STUDY AND RESPONSE TO THERAPY

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Summary: The prevalence of cytomorphologic types of canine lymphoma is described for the first time in Slovenia as well as their response to selected chemotherapeutic protocols.
Fine-needle aspiration was used as routine diagnostic method in attempt to estimate more accurately first remission and survival time in lymphoma affected dogs. In a retrospective study including 39 dogs with lymphoma we have explored the occurrence of different cytomorphologic types of canine lymphoma and evaluated the influence of clinical stage, choice of therapy and sex on survival time of treated dogs. One dog (2.6 %) had a low grade lymphoma classified as the small lymphocytic type, in 13 dogs (33.3 %) intermediate grade lymphoma (mixed, large and small cleaved) were observed, and in 25 dogs (64.1 %) high grade tumours (lymphoblastic, immunoblastic, small noncleaved) were determined. The survival times of the dogs with high-grade tumours were better than of those with low-grade, due to more complete responses to the therapy and longer remission times.
Cytomorphologic evaluation of fine-needle aspirates of affected lymph nodes is suitable for a routine morphological diagnostics, however it can contribute to a more accurate prognosis only in the association with other known prognostic factors.

**Key words:** dog diseases; lymphoma - therapy; biopsy, needle; cytodiagnosis; survival rate; dogs

## Introduction

Malignant lymphoma is a common lymphoproliferative diseases and the third most common malignant tumour in the dog (1). The aetiology is multifactorial, however the factors involved in the development of neoplasm in dogs are poorly understood. Predisposing factors in oncogenesis include genetic background, age, sex, diet, environment, immune response, and stress. Initiation factors, such as oncogenic viruses, chemicals, exposure to various radiation or magnetic fields combined with the susceptibility, can induce genetic mutation of a cell within a tissue, which then undergoes uncontrolled proliferation (2).

Lymphoma can be classified according to the anatomic location, clinical stage, immunofenotype, cytomorphologic type, and finally on the malignancy grade (3). Multicentric form is present in 80 % of the subjects and clinically appears as painless lymphadenomegaly of one or more peripheral lymph nodes (4). Alimentary lymphoma is the second most common, followed by extranodal and mediastinal form (1). Among all histo-cytomorphological classification schemes, the National Institutes of Health (NIH) Working Formulation proved to be the most convenient for determining the type of canine lymphoma: it divides tumours into three major categories applying to the malignancy grade – low, medium, and high – which are subdivided according to the cytomorphologic type. The majority (80 %) of lymphomas in dogs are either of medium or high malignancy grades (5).

Immunophenotyping, sub-classifying lymphomas into B- or T- cell lineage, can significantly lighten the disease prognosis. Approximately 80 % of canine multicentric lymphomas are B-cell, the

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remaining 20 % comprised of T-cell lineage, which have poorer prognosis (6, 7, 8).

The treatment of choice for lymphoma is a combined chemotherapy. Different treatment protocols exists, however in the majority of them a combination of vincristine (Oncovin®-O), cyclophosphamide (C), doxorubicin (Adriablastin®-A), prednisolone (P), L-asparginase is used (5). Regardless of the protocol, approx. 70-80 % of dogs respond with complete or partial remission in the duration of 6-9 months (2). Overall recover response rates of 40-50 % are reported. However, most responses are not long-lasting, with median duration of 1.5-2 months being usual (5). Overall survival period for lymphoma affected dogs is 9 – 12 months, although survival periods longer than 14 months have been reported (1). Poor prospects in most cases are mainly the reason that the owners decided for euthanasia of the dogs after the first remission. For this reason the remission and survival time of lymphoma affected dogs are difficult to evaluate.

This is the first study in Slovenia, in which the prevalence of cytomorphologic types of canine lymphoma is described as well as their response to the treatment with the selected chemotherapeutic protocols.

## Material and methods

In all thirty-nine dogs which were included in our study, enlarged lymph nodes were observed during routine clinical approach. Their clinical condition was determined by physical examination, radiographic investigation of thoracic cavity, ultrasound examination of abdomen, and complete blood count.

Cytological samples of enlarged prescapular and/or popliteal lymph nodes were obtained with a fine needle aspiration. Air-dried smears were stained with Giemsa and cytomorphologically evaluated with the microscope Nikon Microphot FX-A (Nikon Instruments Europe BV, Badhoevedorp, The Netherlands) and the Lucia-G image analyzing system (Laboratory Imaging, Prague; Czech Republic). Cytomorphologic type of lymphoma was determined by observing 7 fields at 40x objective magnification following the NIH Working Formulation criteria. We have estimated the following criteria: nuclear size and shape, presence of nucleoli, and mitotic rate (low mitotic index defined as 0 mitoses per field, medium as 1 per field and high as 2 or more mitotic figures per field). In the 13 treated dogs also the remission time or survival rate has been correlated according to the cytomorphological type, malignancy grade, and sex.

The percentages of each cytomorphological type and malignancy grade in all the 39 included dogs and median first remission and survival time in the 13 treated dogs were calculated.

## Results

In the 39 examined dogs only one (2.6 %) had a small lymphocytic type, a low grade tumour. Tumours of intermediate malignancy grade were observed in 13 dogs (33.3 %): in 4 (10.2 %) the mixed (Fig. 1), in 7 (18 %) the large (Fig. 2) and in two (5.1 %) the small cleaved lymphoma (Fig. 3). In 25 cases (64.1 %) the high grade tumors were determined: in 13 dogs (33.3 %) the lymphoblastic (Fig. 4), in 7 (18 %) the immunoblastic (Fig. 5) and in 5 (12.8 %) the small non-cleaved cytological type (Fig. 6) with mostly high mitotic index.

The first remission time in 13 dogs which were treated with different chemotherapeutic protocols was evaluated. Regardless of the cytomorphological type and malignancy grade, 6 dogs were treated with COP protocol, 4 were treated with COPA protocol, 2 with doxorubicin, and 1 with metilprednisolone only. Median survival for COP-treated dogs was 160 days (60-270 days), while the COPA-treated dogs achieved median survival time 225 days (240-270 days). Both dogs, treated with doxorubicin alone, lived 90 days after the start of treatment. One dog, given metilprednisolone alone, lived 240 days, despite the high malignancy and advanced stage of the tumour.

Considering malignancy grade, regardless of the chemotherapeutic protocol used the overall first remission/survival time for low malignant lymphoma was 90 days, for medium 190 days (90-270 days) and for high malignant tumours 195 days (90-270 days).

Among the treated dogs, of which there were 7 males and 6 females, we have noticed a longer first remission rate in females. Median first remission time was 159 days (60-270 days) in male dogs, while 215 days (90-270 days) in females.

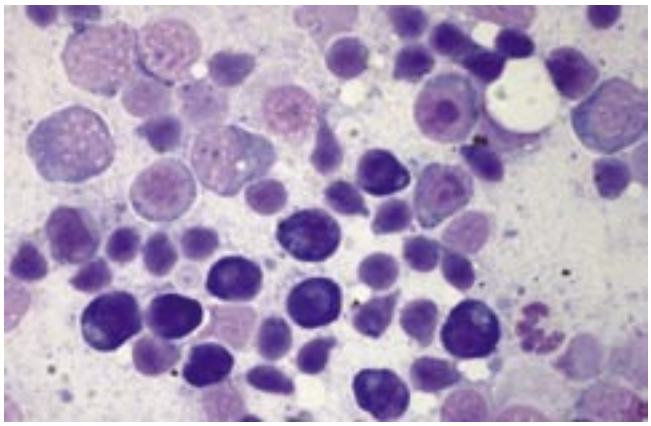

**Figure 1:** Mixed lymphoma. The specimen demonstrates the coexistence of two distinct sizes of nuclei. Giemsa. X40

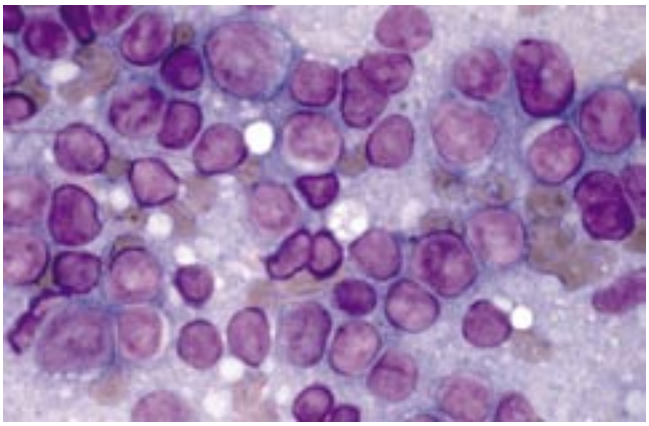

**Figure 2:** Large lymphoma. Note the large size of noncleaved nuclei. Giemsa X40

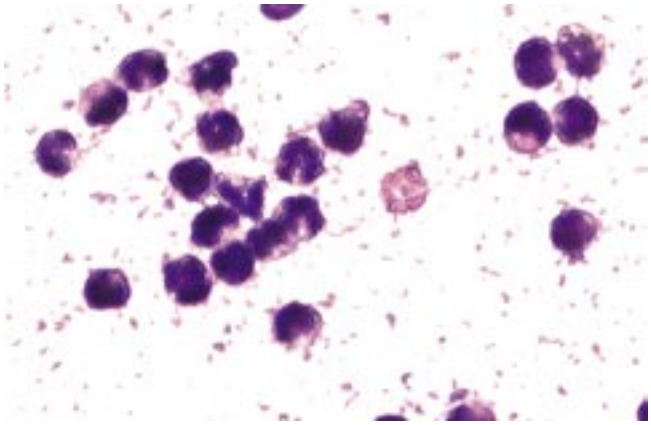

**Figure 3:** Small cleaved lymphoma. Dense, hyperchromatic nuclei with deep linear indentations are prominent. Giemsa. X40

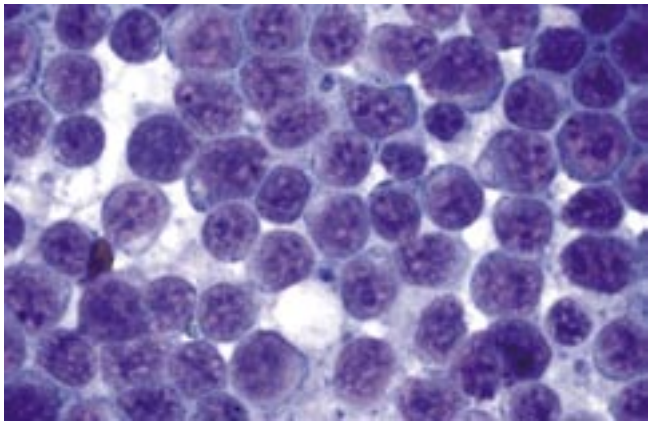

**Figure 4:** Lymphoblastic lymphoma. The extremely irregular outlines of the nuclei are evident, while the cytoplasm is almost undetectable. Giemsa. X40

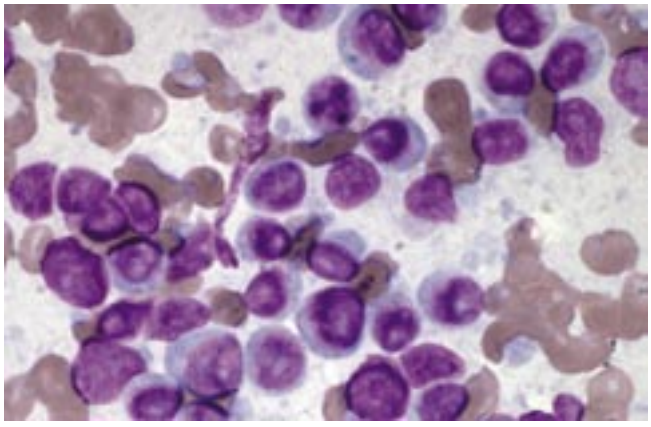

**Figure 5:** Immunoblastic lymphoma. Note the plasmacytoid type of neoplastic cells with nuclei of about 1.5 to 2 red blood cells in diameter. Giemsa. X40

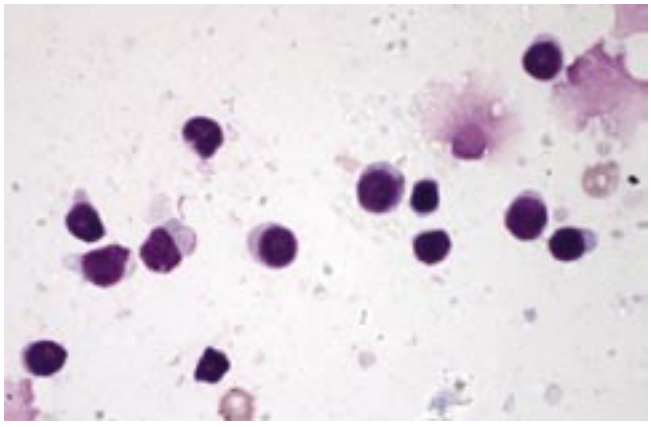

**Figure 6:** Small noncleaved lymphoma. A homogenous population of round nuclei with complete rings of cytoplasm. Giemsa X40

**Table 1:** The results of the cytomorphological examination results of canine lymphoma according to the NIH Working Formulation criteria

| Case No. | Median nuclear size (µm) | | Nuclear shape | Presence and location of nucleoli | Cytoplasm | No. of mitotic figures per field | Mitotic index |
|---|---|---|---|---|---|---|---|
| LOW GRADE | | | | | | | |
| Small lymphocytic | | | round | absent | plasmacytoid type | | |
| 1 | 8.01 | | | | | 0 | low |
| INTERMEDIATE GRADE | | | | | | | |
| Small cleaved | | | angular | not prominent | scant | | |
| 2 | 7.12 | | | | | 0 | low |
| 3 | 6.47 | | | | | 0 | low |
| Large | | | mostly round | multiple peripheral | mostly plasmocytoid type | | |
| 4 | 14.32 | | | | | 3 | high |
| 5 | 12.89 | | | | | 2 | high |
| 6 | 14.47 | | | | | 1 | medium |
| 7 | 13.25 | | | | | 2 | high |
| 8 | 14.76 | | | | | 1 | medium |
| 9 | 13.21 | | | | | 2 | high |
| 10 | 12.68 | | | | | 2 | high |
| Mixed | | | angular (s) | not prominent (s) | scant (s) | | |
| | s | l | round (l) | multiple peripheral (l) | prominent (l) | | |
| 11 | 6.53 | 13.92 | | | | 0 | low |
| 12 | 5.76 | 14.64 | | | | 0 | low |
| 13 | 5.88 | 14.01 | | | | 0 | low |
| 14 | 6.92 | 12.58 | | | | 1 | medium |
| HIGH GRADE | | | | | | | |
| Immunoblastic | | | oval | single central larg | plasmacytoid type | | |
| 15 | 8.65 | | | | | 2 | high |
| 16 | 9.05 | | | | | 0 | low |
| 17 | 9.37 | | | | | 2 | high |
| 18 | 11.21 | | | | | 3 | high |
| 19 | 10.34 | | | | | 0 | low |
| 20 | 12.18 | | | | | 1 | medium |
| 21 | 10.72 | | | | | 3 | high |
| Lymphoblastic | | | angular | not prominent | not prominent | | |
| 22 | 9.62 | | | | | 2 | high |
| 23 | 9.80 | | | | | 2 | high |
| 24 | 10.58 | | | | | 3 | high |
| 25 | 8.85 | | | | | 2 | high |
| 26 | 9.34 | | | | | 1 | medium |
| 27 | 10.05 | | | | | 2 | high |
| 28 | 8.75 | | | | | 0 | low |
| 29 | 9.44 | | | | | 1 | medium |
| 30 | 9.82 | | | | | 0 | low |
| 31 | 11.28 | | | | | 2 | high |
| 32 | 9.98 | | | | | 2 | high |
| 33 | 10.33 | | | | | 3 | high |
| 34 | 11.02 | | | | | 1 | medium |
| Small non-cleaved | | | uniformly round | not prominent | forms a complete ringe around nucleus | | |
| 35 | 8.23 | | | | | 3 | high |
| 36 | 7.59 | | | | | 2 | high |
| 37 | 6.97 | | | | | 0 | low |
| 38 | 8.05 | | | | | 1 | medium |
| 39 | 7.5 | | | | | 2 | high |

Legend: (s) – small cells, (l) – large cells

**Table 2:** Survival rate of 13 treated dog with multicentric lymphoma according clinical stage and substage, and used chemotherapeutic protocol

| No | Case No. | Sex | Breed | Stage and substage | Therapy | Survival rate (days) |
|---|---|---|---|---|---|---|
| LOW GRADE | | | | | | |
| Small lymphocytic | | | | | | |
| 1 | 1 | M | ECS | III a | COP | 90 |
| INTERMEDIATE GRADE | | | | | | |
| Large | | | | | | |
| 2 | 4 | F | RW | III a | COPA | 240 |
| 3 | 5 | M | GR | III b | COP | 270 |
| 4 | 7 | M | MMS | I a | DOX | 90 |
| 5 | 9 | F | RW | III b | COP | 180 |
| Mixed | | | | | | |
| 6 | 11 | F | X | II a | COPA | 270 |
| 7 | 12 | F | MS | II a | DOX | 90 |
| HIGH GRADE | | | | | | |
| Immunoblastic | | | | | | |
| 8 | 15 | M | X | I a | COPA | 240 |
| 9 | 16 | M | RW | III a | COP | 210 |
| Lymphoblastic | | | | | | |
| 10 | 22 | M | BBT | III a | COP | 150 |
| 11 | 27 | F | KS | III a | M* | 240 |
| 12 | 28 | F | B | I a | COPA | 270 |
| 13 | 30 | M | GH | III b | COP | 60 |

Legend: euth. - euthanasia, M* - metilprednisolone
Breeds: ECS-English Cocker Spaniel, RW-Rottweiler, GR-Golden Retriever, MMS-Miniature Schnauzer, X-mix breed, MS-Middle Schnauzer, BBT-Old English Sheepdog (bobtail), KS-Karst Sheepherd dog, B–Boxer, GH– German Sheperd dog

## Discussion

The results of cytomorphologic analysis in our study are comparable to the literature data (11, 12, 13). High malignant lymphoma represented 64.1 % (25/39) of all evaluated samples, 33.3 % (13/39) were intermediate grade lymphomas and 2.6 % (1/39) low malignant lymphomas. The prevalence of certain cytomorphologic types in our group of patients was comparable to the ones Carter et al. (1986) reported in their work. Beside the small lymphocytic lymphoma there are two other types which are also of low malignancy. Because they usually have a follicular pattern of appearance in the lymph nodes, their determination by fine-needle aspirates is unsuitable. This fact probably influenced on lower prevalence of low malignant lymphoma in our study in comparison to others (12). In addition, neoplastic cells in follicular small cleaved and follicular mixed lymphoma are same as in their diffuse types, which are of medium malignancy, and therefore the error could be even greater, when lymphomas are classified according to the malignancy grade. In an extensive retrospective study of 285 cases only two dogs (0.8 %) have had a follicular lymphoma (11). It is possible that some of the diffuse canine lymphomas initially had been follicular and have progressed to the more aggressive diffuse tumours by the time the animal underwent diagnostic biopsy. Similar progression has been reported in human lymphoma (13).

The prognosis for dogs with lymphoma is variable and depends on a number of factors. High and medium grade lymphomas are associated with high response rate to chemotherapy but reduced survival duration in contrast to low grade lyphomas, which seems to be controversy. T-cell phenotype reduced response and survival durations. Hypercalcaemia is a negative factor if associated with T-cell subtype and reduced renal function, witch mainly leads to shorter survival time. Also the P-glycoprotein expression may be associated with poor response rates and shorter remission. Some studies suggest that females have a more favourable prognosis and that prolonged steroid pre-treatment can reduce

response duration. AgNOR, PCNA, and Ki67 used as prognostic factors are contradictory (5). Cranial mediastinal lymphadenopathy can result in shorter remission and survival duration and finally leukaemia and diffuse cutaneous or alimentary forms of lymphoma can make the prognosis worse (5).

Different studies report that the overall median duration of remission ranges from 45 – 334 days, and the mean duration of remission ranges from 129 – 184 days, with a 58 % - 96 % complete remission rate in dogs treated with chemotherapy (1). The median survival times for dogs with lymphoma treated with chemotherapy are reported to range from 112 – 357 days (1, 5), with 25 % of patients surviving for 2 years (5). Greenlee et al. (1990) stated that survival times of the dogs with high-grade tumours are, paradoxally, better than of those with low-grade lymphomas, because of more complete responses to the therapy and longer remission times. Similar results were obtained in our study, in which the first remission period / survival time of the dog with a low malignant lymphoma was 90 days, 159 days (90-270 days) in those with intermediate grade, while the median remission and survival of dogs with high grade tumours was 255 days (240-270 days).

Advances in remission and survival duration may occur with the development of new chemotherapeutic drugs or novel treatment modalities. Mechanisms of avoiding multidrug resistance, enhancing tumour apoptosis, targeting treatments with immunoconjugates, i.e. antibody-directed therapies, and novel immunomodulatory and radio-therapy based therapies are all active areas of investigation in both human and veterinary medicine (5). Immunotherapy in combination with chemotherapeutic protocols has been used in attempt to achieve longer remissions and survival times. In the immunotherapy specific monoclonal antibodies developed against tumour antigens are used. Development of a monoclonal antibodies from canine lymphoma cell line started about 20 years ago. The antibody CL/Mab231 (Synbiotics Corp, USA) which mediates antibody-dependent cell cytotoxicity is successfully used as an adjuvant during maintenance phase of the chemoimmunotherapy with intention to destroy residual remaining tumour cells (1). Unfortunately most of these new treatments still remain unavailable for routine clinical use, predominantly because of high price.

Statistical evaluation of the correlation between the cytomorphologic type of lymphoma and remission or/and survival rate could not be provided due to small number of patients and different treatment protocol used. According to the literature, despite repeated considerations in the past, no strong correlation has been shown between cytomorphologic type of lymphoma and either remission rate or survival (11).

We can conclude that the cytomorphologic evaluation of fine-needle aspirates of affected lymph nodes is suitable for a routine diagnosis; however it can contribute greatly to a more accurate prognosis only in association with all other known prognostic factors. Further studies should be performed to confirm if cytomorphological characteristic of the lymphoma could be used as a reliable prognostic factor.

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## LIMFOM PRI PSU: CITOLOŠKE ZNAČILNOSTI IN ODGOVOR NA ZDRAVLJENJE

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**Povzetek:** Raziskava predstavlja prvo študijo o pojavljanju posameznih citomorfoloških tipov limfoma pri psih v Sloveniji v povezavi s trajanjem odgovora na izbran protokol zdravljenja in dobo preživetja.

Tankoigelno aspiracijsko biopsijo prizadetih bezgavk smo uporabili kot rutinsko diagnostično metodo. V retrospektivni študiji smo pri 39 obolelih psih ugotavljali pojavljanje določenega citomorfološkega tipa limfoma v Sloveniji in vrednotili vpliv kliničnega stanja, izbire terapije in spola na obdobje preživetja pri zdravljenih živalih. Malolimfocitni tip limfoma z nizko stopnjo malignosti je bil ugotovljen le pri enem psu (2,6 %). Pri 13 (33,3 %) so bili ugotovljeni tumorji srednje stopnje malignosti (mešani tip, velikocelični razcepljeni tip, malocelični razcepljeni tip), 25 psov (64,1 %) pa je obolelo za limfomi visoke stopnje malignosti (limfoblastni tip, imunoblastni tip, malocelični nerazcepljeni tip). Daljši čas prve remisije (trajanje odgovora na zdravljenje) in dobo preživetja smo ugotavljali pri psih, obolelih za oblikami limfomov z visoko stopnjo malignosti.

Citomorfološko vrednotenje celičnih razmazov drobnoigelnih aspiratov prizadetih bezgavk se je izkazalo kot primerna metoda za rutinsko diagnostiko tumorjev, vendar pa je za celovitejšo napoved obolenja potrebno upoštevati tudi ostale prognostične dejavnike.

**Ključne besede:** psi, bolezni; limfom - zdravljenje; biopsija aspiracijska; citodiagnostika; doba preživetja; psi

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**Original Research Paper**

# ENDOCRINE AND METABOLIC RESPONSES OF MARWARI SHEEP IN ARID TRACT

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**Summary:** The present investigation was carried out in Marwari sheep belonging to arid tracts to assess the endocrine and metabolic responses during water restriction, hot ambience, road transportation and drought. The mean±SEM values of serum thyroxine (µg/dl), triiodothyronine (ng/dl), aldosterone (ng/dl), cortisol (ng/ml), cholesterol (mg/dl), glucose (mg/dl), sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), total serum protein (g/dl), albumin (g/dl), globulin (g/dl), urea (mg/dl) and creatinine (mg/dl) during control period of water restriction experiment were 4.89±0.04, 123. 1±1.8, 1.79±0.15, 6.89±0.17, 40.2±2.9, 70.3±4.2, 140.1±2.8, 5.0±0.12, 130.3±4.1, 7.4±0.1, 3.7±0.09, 3.5±0.1, 25.5±2.1 and 1.0±0.06, respectively. An increase in glucose, cholesterol, aldosterone, sodium, chloride, total serum protein, albumin, globulin, urea and creatinine concentrations was observed during the water restriction period and decrease in concentrations of the above parameters was seen upon rehydration. Concentrations of thyroxine, triiodothyronine and potassium were decreased during the water restriction period which showed an increase during rehydration. During hot ambience pattern of changes were comparable to those observed during water restriction. A significant ($p \leq 0.05$) increase was observed in endocrine responses after five hours of road transportation. Drought caused an increase in the mean values of aldosterone, cortisol, sodium, chloride, urea and creatinine; no change in glucose and cholesterol and decrease in thyroid hormones, total proteins, albumin and globulin.

**Key words:** acclimatization; desert climate; endocrine system – physiology; stress – physiopathology; body water – metabolism; aldosterone – blood; cortisol – blood; thyroxine - blood; triiodothyronine - blood; sheep

## Introduction

Arid tracts face the problems of recurring droughts and it becomes essential to determine the adaptability level of the animals living in these areas to increase their sustainability by taking proper and timely measures during such periods. The important way to determine physiological response to the external and internal stimuli and an understanding of the physiology of stress in animals is crucial in assessing animal welfare. Examination of the stress and productivity responses is important to develop management strategies (1).

The common problems experienced by the sheep in arid tracts are dehydration, heat, transportation and droughts. When the pressure from these conditions becomes excessive new defense mechanisms are initiated and their determination is an essential part of animal management. Out of all the mechanisms endocrine and metabolic responses are important giving a picture of modulation in physiological mechanisms. The turnover of fluid during dehydration and rehydration shows the adaptation to water deficit and water plus and many endocrinological factors tend to control them. The hypothalamic-pituitary-adrenal axis is important in making the physiological approach to regulate the unusual condition. Increased release of corticotrophin releasing hormone (CRH), adreno-corticotrophin hormone (ACTH) and thyroid stimulating hormone (TSH) during stress are related with increased concentrations of thyroid hormones and adrenal ster-

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oids (1). Adrenal hormone cortisol is equated with the stress level. Another adrenal steroid aldosterone mediates retention of considerable quantities of water along with sodium. Aldosterone is also considered as a hormone of dehydration in animals. It is mainly concerned with sodium retention and potassium excretion into the urine. Cortisol from adrenal cortex exerts some effect on electrolytes and water metabolism (2).

In stressed animals thyroid activity acts to strengthen the body mechanisms. Therefore the thyroidal hormone levels are good indicators of health status of the animals. Determination of serum thyroid hormones along with cholesterol, glucose and proteins is important and correlation of thyroid hormones with cholesterol is studied by few workers in animals (3).Marwari sheep plays an important role in the economy of arid tracts. However, there is paucity of work regarding endocrine and metabolic responses to various types of stress which incited the investigation.

## Material and methods

To determine the endocrine and metabolic responses of Marwari sheep belonging to arid tracts, two experiments were designed which were not related with each other according to selection of animals. The first experiment was conducted to determine endocrine and metabolic responses during water restriction and rehydration in female Marwari sheep which were kept in pens. The second experiment was taken up to assess endocrine and metabolic responses during hot ambience, after road transportation and during drought condition on male sheep belonging to farmers' stock of arid region.

First experiment was carried out on eleven apparently healthy female Marwari sheep (1-2 years) kept in well ventilated pens to provide stress free environment and were fed with roughage diet of dry leaves of Ziziphus nummularia along with pelleted diet (crude protein-20 %, ether extract 2.5 %, crude fibre 12 %, acid insoluble ash 4 %, calcium 0.5 %, phosphorus 0.5 %, vitamin $AD_3$ 5000 IU/kg and mineral mixture 1 %) and watered ad libitum. Before the beginning of experiment animals were accustomed to the new surroundings and environment so that stress or anxiety due to new ambience could be minimized. This experiment was conducted under the permission of Institutional Animal Ethical Committee, College of Veterinary and Animal Science, Bikaner, India. All the measures were taken for the comfort of the animals and health was monitored regularly.

To determine endocrine and metabolic responses of sheep during water restriction and rehydration, the experiment was divided into three periods i.e. control, water restriction and rehydration. In each period same animals were used so that each animal served as its own control. During the control period of 5 days the animals were fed and watered ad libitum. Water restriction period was of 8 days during which water was restricted completely but animals were fed ad libitum. After completion of water restriction period, rehydration period started when animals were fed and watered ad libitum. It was of 5 days. Blood samples were collected to harvest sera in the morning hours before feeding. During control period samples were collected daily for five days and mean was used as a representative value for each parameter. Sampling was carried out in duplicate on day 4 and 8 of water restriction period. During rehydration period samples were collected on hour 1/2, 24, 48, 72 and 96 of rehydration.

Second experiment was conducted on the Marwari sheep which belonged to farmers' stock of arid region. These animals were not kept in the pens and maintained under semi conservative system and were fed and watered ad libitum. The male animals were selected as these animals were kept for meat purposes and small amount of blood was collected as routine health check up. Male animals are generally transported from villages to cities for meat purposes. Therefore to assess the stress to the animals blood samples were collected.

In this experiment, three groups of animals were taken. The first group constituted of 10 male sheep (1-2 years) from which samples (sera) were collected during moderate (maximum temperature varied between 23 and 27 °C) and hot ambience (maximum temperature varied between 43 and 46 °C). The mean values of various parameters determined during moderate ambience were considered as control for comparison.

Second group comprised of another 10 male sheep (1-2 years) from which blood samples (sera)were collected after 5 hours of road transportation and then after four hours of rest when they reached to destination.

Third group comprised of another 10 male sheep (1-2 years) belonging to farmers' stock of drought stricken arid tract.

In all the experiments endocrine responses were assessed by determining serum hormones by radio-

immunoassay (RIA) in Radio Isotope Laboratory, College of Veterinary and Animal Science, Bikaner. $^{125}I$ was used for labeling each hormone. Assay was based on the competition between labeled and unlabelled hormones for the limited binding sites of antibody. Gamma Counter was used to count radioactivity for the determinations. RIA kits were used for hormone determinations viz. thyroxine (RIA kit, DiaSorin), triiodothyronine (RIA kit, DiaSorin), aldosterone (RIA kit, DPC) and cortisol (RIA kit, DPC). The metabolic responses were evaluated by determining parameters in the serum viz. total proteins, albumin, globulin, creatinine, urea and cholesterol by colorimetric kits (Wipro) and glucose (Nelson - Somogyi method),sodium, potassium (Flame photometer), and chloride (Schales and Schales) by the standard methods (4). Statistical significance was assessed by 't' test (5).

## Results

The mean values of endocrine and metabolic responses during water restriction are presented in table 1 and during hot ambience, drought and after road transportation are presented in table 2.

### *1. Endocrine and metabolic responses during water restriction and rehydration*

During water restriction period the mean values of serum $T_4$ and $T_3$ decreased significantly ($p \leq 0.05$) from the respective control mean values. Upon rehydration, at hour 1/2 the mean values of serum $T_4$ and $T_3$ were significantly ($P \leq 0.05$) higher from respective day 8 (water restriction period) mean values. Afterwards the values started increasing as the time advanced and at hour 96 differed non-significantly ($P > 0.05$) from respective control mean values.

The mean values of serum glucose and cholesterol increased significantly ($p \leq 0.05$) during water restriction period. During rehydration period at hour 1/2 both the mean values were significantly ($P \leq 0.05$) lower than the respective day 8 mean values. At hour 72 a non -significant ($p > 0.05$) difference from respective control mean value was observed indicating attainment of normalcy.

As the days during water restriction period advanced the mean values of aldosterone started increasing significantly ($P \leq 0.05$). Upon rehydration aldosterone mean value decreased significantly ($P \leq 0.05$) when compared with that of day 8. At hour 24 and 48 it showed an increase and then again showed a decreasing trend and the mean value touched the base line on hour 72.

With the progress of water restriction period the mean values of serum sodium and chloride started rising while potassium showed a declining trend. Upon rehydration the mean values of serum sodium and chloride lowered significantly ($P \leq 0.05$) when compared with the respective day 8 dehydration mean values. The mean values of all the electrolytes during rehydration phase were compared with the respective control mean values to find out the time period when non-significant ($p > 0.05$) variation could be observed showing normalcy. For sodium it was hour 48, while for potassium and chloride it was hour 72.

During water restriction period serum cortisol increased significantly ($P \leq 0.05$) from control mean value and on day 8 increase was approximately 7-fold of the control value. Immediately after rehydration cortisol mean value marked a significant lowering from day 8 mean value and then onward this trend remained continued until the value touched base level at hour 96.

With the progression of water restriction period an increase was observed in the mean values of serum total proteins, albumin, globulin, urea and creatinine. Upon rehydration a decrease was observed in the mean values of all the parameters. The mean values at hour 1/2 were compared with respective day 8 mean values. All the mean values were significantly ($p \leq 0.05$) lower except for globulin. All the mean values touched the base line at hour 72 except that for serum creatinine which touched the base line at hour 96 of rehydration.

### *2. Endocrine and metabolic responses during hot ambience, after road transportation and drought*

The mean value of each parameter during hot ambience, drought, road transportation and rest were compared with the respective mean values during moderate ambience considering it as control. During hot ambience and drought serum thyroxine and triiodothyronine levels decreased and after road transportation increased significantly ($p \leq 0.05$) when compared from respective control mean values. Serum glucose and cholesterol showed significant ($p \leq 0.05$) increase from respective control mean values during hot ambience and after road transportation. Mean values of both the parameters showed non- significant ($p > 0.05$) changes from control during drought.

**Table 1:** Endocrine and metabolic responses during water restriction in Marwari sheep (N=11)

| Parameters (Mean±SEM) | Experimental periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Water restriction (Days) | | Rehydration (Hours) | | | | |
| | | 4 | 8 | 1/2 | 24 | 48 | 72 | 96 |
| Thyroxine µg/dl | 4.89±0.04 | 3.8±0.09$^{b}$ | 2.1±0.07$^{b}$ | 2.6±0.07$^{bh}$ | 2.9±0.08$^{b}$ | 3.4±0.08$^{b}$ | 3.9±0.09$^{b}$ | 4.6±0.10$^{a}$ |
| Triiodothyronine ng/dl | 123.1±1.8 | 112±1.2$^{b}$ | 75.2 ±2.0$^{b}$ | 83±1.17$^{bh}$ | 100±1.01$^{b}$ | 110±1.1$^{b}$ | 116±1.5$^{b}$ | 125±1.07$^{a}$ |
| Aldosterone ng/dl | 1.79±0.15 | 3.8±0.19$^{b}$ | 6.0±0.17$^{b}$ | 2.0±0.13$^{bh}$ | 2.4±0.18$^{b}$ | 2.6±0.18$^{b}$ | 1.9±0.19$^{a}$ | 1.6±0.10$^{a}$ |
| Cortisol ng/ml | 6.89±0.17 | 13.8±1.19$^{b}$ | 43.1±1.27$^{b}$ | 20.6±0.17$^{bh}$ | 16.9±2.18$^{b}$ | 12.4±1.98$^{b}$ | 9.9±1.19$^{b}$ | 7.0±0.9$^{a}$ |
| Glucose mg/dl | 40.2±2.9 | 49.2±2.9$^{b}$ | 69.2±2.9$^{b}$ | 52.2±2.9$^{bh}$ | 48.2±3.1$^{b}$ | 45.9±2.1$^{b}$ | 43.1±2.4$^{a}$ | 42.1±3.1$^{a}$ |
| Cholesterol mg/dl | 70.3±4.2 | 81.3±4.0$^{b}$ | 110.3±3.9$^{b}$ | 89.3±4.1$^{bh}$ | 85.3±3.0$^{b}$ | 80.3±3.3$^{b}$ | 74.3±4.1$^{a}$ | 73.0±3.2$^{a}$ |
| Sodium mmol/l | 140.1±2.8 | 152±1.8$^{b}$ | 175.2 ±2.0$^{b}$ | 153±1.17$^{bh}$ | 150±1.01$^{b}$ | 145±1.1$^{a}$ | 141±1.5$^{a}$ | 139±1.07$^{a}$ |
| Potassium mmol/l | 5.0±0.12 | 4.5±0.13$^{b}$ | 3.4±0.11$^{b}$ | 3.9±0.2$^{bh}$ | 4.2±0.1$^{b}$ | 4.7±0.1$^{b}$ | 4.9±0.2$^{a}$ | 5.4±0.1$^{a}$ |
| Chloride mmol/l | 130.3±4.1 | 138.3±3.0$^{b}$ | 170.3±3.9$^{b}$ | 153.3±4.1$^{bh}$ | 148.3±3.0$^{b}$ | 140.3±3.3$^{b}$ | 136.3±4.1$^{a}$ | 133.0±3.2$^{a}$ |
| Total proteins g/dl | 7.4±0.1 | 8.2±0.1$^{b}$ | 10.2 ±0.2$^{b}$ | 9.0±0.17$^{bh}$ | 8.5±1.01$^{b}$ | 8.2±0.6$^{b}$ | 7.8±0.2$^{a}$ | 7.6±0.4$^{a}$ |
| Albumin g/dl | 3.7±0.09 | 4.3±0.08$^{b}$ | 5.3±0.07$^{b}$ | 4.2±0.09$^{bh}$ | 4.0±0.05$^{b}$ | 3.8±0.05$^{b}$ | 3.5±0.04$^{a}$ | 3.1±0.01$^{a}$ |
| Globulin g/dl | 3.5±0.1 | 4.3±0.3$^{b}$ | 4.6±0.1$^{b}$ | 4.8±0.1$^{b}$ | 4.3±0.3$^{b}$ | 4.1±0.3$^{b}$ | 3.7±0.1$^{a}$ | 3.5±0.2$^{a}$ |
| Urea mg / dl | 25.5±2.1 | 37.5±2.2$^{b}$ | 55.8±2.5$^{b}$ | 40.5±3.1$^{bh}$ | 34.5±2.4$^{b}$ | 30.2±2.6$^{b}$ | 28.6±2.2$^{a}$ | 26.3±3.1$^{a}$ |
| Creatinine mg / dl | 1.0±0.06 | 3.5±0.06$^{b}$ | 4.9±0.05$^{b}$ | 2.9±0.07$^{b}$ | 2.0±0.03$^{b}$ | 1.7±0.02$^{b}$ | 1.5±0.03$^{b}$ | 1.1±0.06$^{a}$ |

N=Number of animals

The mean value of each parameter in each period has been compared with the respective mean value during control period. Significant variation ($p \leq 0.05$) has been shown by using superscript 'b' and non-significant ($p > 0.05$) by using 'a'.

Superscript 'h' on the mean value of a parameter at hour 1/2 shows significant ($p \leq 0.05$) difference from the respective day 8 (water restriction period) mean value.

Serum aldosterone was significantly ($p \leq 0.05$) higher during hot ambience, drought and after road transportation from control mean value. Serum sodium and chloride were significantly ($p \leq 0.05$) higher during hot ambience only and non significantly ($p > 0.05$) higher during drought and after road transportation. Serum potassium was significantly ($p \leq 0.05$) lower during hot ambience, drought and after road transportation.

During hot ambience serum cortisol, total proteins, albumin, globulin, urea and creatinine showed a significant ($p \leq 0.05$) increase. After road transportation only serum cortisol increased significantly ($p \leq 0.05$). During drought condition cortisol, urea and creatinine showed a significant ($p \leq 0.05$) increase while total proteins, albumin and globulin showed significant ($p \leq 0.05$) decrease. All the parameters showed non significant ($p > 0.05$)

**Table 2:** Endocrine and metabolic responses in Marwari sheep during hot ambience, drought and after road transportation

| Parametrs (Mean±SEM) | Control (Moderate ambience ) (10)* | Hot ambience (10)* | Drought (10) | Road transportation (10)** | Rest Period (10)** |
|---|---|---|---|---|---|
| Thyroxine µg/dl | 4.9±0.05 | 2.6±0.09$^{b}$ | 2.5±0.11$^{b}$ | 7.2±0.2$^{b}$ | 5.2±0.05$^{a}$ |
| Triiodothyronine ng/dl | 120.6±1.3 | 101±1.3$^{b}$ | 100.3±1.1$^{b}$ | 130.2 ±2.9$^{b}$ | 123.3±1.2$^{a}$ |
| Aldosterone ng/dl | 1.66±0.11 | 4.8±0.16$^{b}$ | 4.9±0.2$^{b}$ | 2.7±0.15$^{b}$ | 1.7±0.10$^{a}$ |
| Cortisol ng/ml | 6.83±0.17 | 20.0±1.7$^{b}$ | 26.0±0.19$^{b}$ | 40.6±3.1$^{b}$ | 7.99±0.13$^{a}$ |
| Glucose mg/dl | 42.8±3.2 | 57.5±3.3$^{b}$ | 42.6±0.29$^{a}$ | 53.2±3.2$^{b}$ | 52.8±3.2$^{b}$ |
| Cholesterol mg/dl | 74.7±5.3 | 90.1±3.9$^{b}$ | 72.1±4.09$^{a}$ | 70.3±4.1$^{a}$ | 70.7±5.0$^{a}$ |
| Sodium mmol/l | 137.1±2.3 | 165±1.6$^{b}$ | 142.6±5.09$^{a}$ | 146±1.3$^{a}$ | 133.1±2.9$^{a}$ |
| Potassium mmol/l | 5.2±0.11 | 4.0±0.12$^{b}$ | 4.6±0.19$^{b}$ | 4.5±0.1$^{b}$ | 5.5±0.19$^{a}$ |
| Chloride mmol/l | 133.3±3.1 | 158.3±3.5$^{b}$ | 140.2±5.01$^{a}$ | 141.3±3.1$^{a}$ | 136.3±3.0$^{a}$ |
| Total proteins g/dl | 7.4±0.1 | 8.2 ±0.3$^{b}$ | 6.0±0.1$^{b}$ | 7.8±0.2$^{a}$ | 7.6±0.1$^{a}$ |
| Albumin g/dl | 3.7±0.09 | 4.3±0.08$^{b}$ | 3.0±0.02$^{b}$ | 3.8±0.09$^{a}$ | 3.7±0.1$^{a}$ |
| Globulin g/dl | 3.5±0.1 | 3.9±0.2$^{b}$ | 3.0±0.03$^{b}$ | 3.4±0.1$^{a}$ | 3.1±0.1$^{a}$ |
| Urea mg / dl | 25.5±2.1 | 40.1±2.1$^{b}$ | 56.0±4.1$^{b}$ | 24.5±3.0$^{a}$ | 21.5±2.0$^{a}$ |
| Creatinine mg / dl | 1.0±0.06 | 1.7±0.03$^{b}$ | 3.6±0.1$^{b}$ | 1.2±0.05$^{a}$ | 0.8±0.02$^{a}$ |

Figures in parentheses indicate number of animals

* and ** = Same animals, respectively

The mean value of each parameter in each effect has been compared with the respective mean value of control. Significant variation ($p \leq 0.05$) has been shown by using superscript 'b' and non-significant ($p > 0.05$) by us 'a'.

changes after rest except glucose when compared from control.

## Discussion

### *1. Endocrine and metabolic responses during water restriction and rehydration*

The decrease in serum T4 and T3 during water restriction period could be related with dearth of feed and water as thyroid hormones elevate basal metabolic rate and heat production for thermoregulation (3). Therefore reduction was beneficial in terms of reducing water losses with each respiration and thereby decreasing oxygen consumption and helped the animal to conserve water. The decreased thyroid activity was related with increased serum cholesterol values (3). Suppression of thyroid activity reduced energy metabolism resulting in higher blood energy nutrient levels (6).

Increased serum aldosterone level during water restriction period (7) probably helped the animals to withstand dehydration stress by water and sodium retention (8). Higher aldosterone values are related to stress (9). In addition to anti diuretic hormone, aldosterone is also important in regulating the body's

water balance. Serum aldosterone was also used as a marker of water and electrolyte homeostasis in sheep (10).

After rehydration decrease in aldosterone mean value was probably due to dilution effect (2). In present study at hour 24 and 48 aldosterone showed an increasing trend followed by a decrease. Earlier literature has also reported an increase in serum aldosterone upon rehydration in sheep (11, 12).

With the progress of water restriction period the mean values of serum sodium and chloride started rising while potassium showed a declining trend (13). Hypernatraemia helped the dehydrated sheep to conserve water by holding it in water compartments and circulation. Decrease in serum potassium during dehydration indicated towards the role of aldosterone on increasing excretion rates of potassium (2).

Upon rehydration the mean values of serum sodium and chloride lowered significantly ($P \leq 0.05$) when compared with the respective day 8 dehydration mean values. The decreased values indicated towards haemodilution. At hour 1/2 slight increase in the mean value of potassium could be due to lowering of aldosterone levels.

Increased serum cortisol during water restriction period indicated towards stress (14) which worked through CRH and ACTH and was essential to supply glucose by gluconeogenesis and glycogenolysis. A rise was observed in the serum glucose. Upon re hydration serum cortisol started declining and touched the base line at hour 96 as observed by earlier workers also (2).

With the progression of water restriction period an increase was observed in the mean values of serum total proteins, albumin, globulin, urea and creatinine which probably helped the animals to hold the water. An increase in serum proteins during dehydration have also been reported in goats (15). Uraemia helped the animals to hold the water during dehydration which resulted due to the combined effect of absorption and reabsorption from the kidneys and alimentary tract (16). Increased creatinine levels during dehydration could be due to decrease in urine output or glomerular filtration rate. Cortisol through muscle wasting was related with increased serum creatinine during dehydration.

### *2. Endocrine and metabolic responses during hot ambience, after road transportation and drought*

During hot ambience and drought serum thyroxine and triiodothyronine levels decreased probably to decelerate basal metabolic rate as a part of thermoregulation. In sheep thyroids are more active during winter months (17). Decreased thyroid activity during hot ambience could also be related with increased concentrations of energy metabolites (glucose and cholesterol) in the serum. However, decreased thyroid activity during drought was not related with the increased concentrations of glucose and cholesterol as there was non significant ($p > 0.05$) difference from control. Increased thyroid activity after road transportation did not bring significant change in the levels of cholesterol. However, serum glucose showed significant rise.

During hot ambience serum cortisol, total proteins, albumin, globulin, urea and creatinine showed a significant increase as reported by earlier workers (18). After road transportation only serum cortisol increased significantly, however, it did not bring significant ($p \leq 0.05$) changes in the levels of metabolites like total proteins, cholesterol, urea and creatinine but serum glucose was significantly ($p \leq 0.05$) higher despite of higher thyroid activity. This indicated towards glucocorticoid function. Cortisol response of animal was faster than the body responses to bring about the resultant alterations in metabolic concentrations except serum glucose which showed immediate response. It was interesting to note that rise in serum cortisol after road transportation was higher than drought but there was no decrease in total serum proteins in former as was observed in latter. This could be explained on the basis that drought conditions have scarcity of water and feed resulting in long term effect of cortisol while transportation appeared to be short term stress to animals. Further, very high creatinine concentration during drought support the explanation. Effect of hot ambience, transportation and drought appeared to be similar on serum aldosterone, sodium, potassium and chloride. However, rise in aldosterone concentration during hot ambience and drought was double than that observed due to transportation.

From the investigation it was concluded that endocrine and metabolic responses elicited during various conditions were variables in animals. Changes in endocrine and metabolic responses due to water restriction were great but reversible. After

elimination of stressor the homeostasis was obtained. After transportation changes in endocrine responses were faster than metabolic responses. Sampling after four hours of rest showed decrease in the levels of all the hormones with non- significant ($p>0.05$) change in serum glucose level.

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## ENDOKRINI IN PRESNOVNI ODZIVI PRI OVCAH PASME MARVARI V SUŠNIH PODROČJIH

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**Povzetek:** Raziskavo smo opravili na ovcah pasme marvari, ki živijo v sušnih področjih. Zanimali so nas presnovni in endokrini odzivi na pomanjkanje pitne vode, visoke temperature, cestni transport in splošno sušo. Med poskusom omejevanja pitne vode so bile izmerjene vrednosti pri kontrolni skupini (povprečje ± SEM) serumskega tiroksina (µg/dl), trijodtironina (ng/dl), aldosterona (ng/dl), kortizola (ng/ml), holesterola (mg/dl), glukoze (mg/dl), natrija (mmol/l), kalija (mmol/l), klorida (mmol/l), skupnih serumskih proteinov (g/dl), albumina (g/dl), globulina (g/dl), ureje (mg/dl) in kreatinina (mg/dl) 4,89 ± 0,04, 123,1 ± 1,8, 1,79 ± 0,15, 6,89 ± 0,17, 40,2 ± 2,9, 70,3 ± 4,2, 140,1 ± 2,8, 5,0 ± 0,12, 130,3 ± 4,1, 7,4 ± 0,1, 3,7 ± 0,09, 3,5 ± 0,1, 25,5 ± 2,1 in 1,0 ± 0,06, v enakem zaporedju. Pri omejevanju pitne vode smo ugotovili povečanje koncentracije serumske glukoze, holesterola, aldosterona, klorida, skupnih serumskih proteinov, albumina, globulina, ureje in kreatinina, po rehidraciji pa so koncentracije navedenih parametrov spet upadle. Koncentracije tiroksina, trijodtironina in kalija so bile med omejevanjem vode znižane, po rehridraciji pa so se zvišale. Podobne spremembe kot pri omejevanju vode smo ugotovili pri skupini ovc, ki so bile nastanjene pri višji temperaturi okolice. Izrazit porast endokrinega odziva ($p \leq 0.05$) smo ugotovili tudi po petih urah prevoza po cesti. Suša je povzročila povečanje srednjih serumskih vrednosti aldosterona, kortizola, natrija, klorida, ureje in kreatinina, zmanjševanje koncentracije hormonov ščitnice, skupnih proteinov, albuminov in globulinov, ni pa bilo sprememb v ravneh glukoze in holesterola.

**Ključne besede:** aklimatizacija; puščavska klima; endokrinisistem - fiziologija; stres - patofiziologija; telesne tekočine - metabolizem; aldosteron - kri; hortizol - kri; tiroksin - kri; trigotironin - kri; ovce

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**Case Report**

# CASE REPORT OF A PSEUDORABIES (AUJESZKY'S DISEASE) IN A BITCH

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**Summary:** Bitch named Neca, mixed breed, spayed, 2,5 years old, was admitted to the Small animal clinic of Veterinary faculty of Ljubljana on 3rd January 2006 at 10.45 a.m. It had no chronic illnesses and was vaccinated against rabies. The day before the clinical signs appeared, it was apparently healthy. During the night it was kept in a garage. From the morning on it has been crying, curling up and foaming at the mouth. It was hospitalised. In spite of intensive care the bitch died 10 hours after admittance to the hospital. Directed questioning of the owners revealed the information that she was fed raw pork 7 days ago. Based on the history and clinical signs, suspected diagnosis of pseudorabies was established. Pathomorphological examination revealed lesions characteristic for pseudorabies, while etiological diagnosis was confirmed by bioassay with rabbit and virus isolation.

**Key words:** dog diseases; pseudorabies – etiology – diagnosis – pathology; herpesvirus 1, suid – isolation and purification; biological assay; dogs

## Introduction

Pseudorabies (Aujeszky's disease, mad itch or infectious bulbar paralysis; AD) is by definition caused by DNA virus belonging to the alpha-herpesvirus family. Infection occurs in most countries of the world with the exception of Australia. Although many mammalian species are susceptible to infection with pseudorabies virus, it represents a predominant problem in pigs which are the main reservoir of the virus. In pigs, the infection is usually subclinical because they have become well adapted to the virus. The disease is usually spreading by commercial movement of infected pigs or contaminated pork products, showing that pseudorabies infection in dogs and cats usually occurs in areas where the disease is enzootic in pigs. Pets infect themselves by consuming contaminated raw pork meat, direct spread from dog to dog has not been described (1). Man and tailless apes seem to be resistant against infection (2).

After ingestion the virus enters nerve endings in the mucosa and spreads to the brain along nerve axons. Inflammation and functional abnormalities in brain cells result in neurological signs after an incubation period of around 3 to 6 days.

The infection of non-adapted species (like dogs are) results with death within hours after showing of the first symptoms (3).

## Clinical case

The dog that has been examined at the Small animal clinic of the Veterinary faculty of Ljubljana was a 2,5 year old, mixed breed bitch, weighting 47,5 kg, vaccinated against rabies and spayed. The owners did not know what could have happened to the dog because it was kept in a garage during the night and that particular morning it could not get up, making strange sounds, the saliva was drooling down her mouth. Although the dog moved freely around the

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owner's estate, they thought it was very unlikely that it would eat something strange. Physical exam revealed dog's inability of standing upright. It was laying on it's side, the temperature was normal (38,0 °C), pulse was strong and regular (130 beats per minute), her CRT was less than 1 second and the color of the mouth mucosa was intensely red. Eyes were bright and the dog seemed to be alert at the time of the first examination. The skin around the neck was scratched and the bitch continued to scratch herself also during the physical exam. The abdomen was soft at palpation and there was no pain revealed.

CBC (complete blood count), biochemical profile, urinary profile and X-ray examination were performed. X-ray examination revealed enlarged urinary bladder and gastritis; thorax was without any pathological signs. Urinary profile was normal except high pH of 8 (normal < 7), slight proteinuria of 1,0 g/L and traces of urobilinogen (3,2 μmol/L). CBC showed $25{,}28\times10^9$ /L WBC (normal range 6,0-$18{,}0\times10^9$ /L), high HCT of 0,60 L/L (normal range 0,35-0,55 L/L), high HGB of 200 g/L (normal range 115-180 g/L). Neutrophilia of 91,1 % (normal range 60-80 %) and lymphopenia of 5,2 % (normal range 12,0-35,0 %) were found in differential. Biochemical parameters have shown hyperglicaemia of 14,8 mmol/L (normal range 3,9-5,0) and low potassium level of 2,95 mmol/L (normal range 3,6-5,0 mmol/L).

Initial therapy consisted of urinary catheter, i/v infusion with Ringer-lactate, antibiotics i/v (amoxycilin+ clavulonic acid in a dosage of 20 mg / kg BID) and i/m (gentamicinum in a dosage of 4 mg / kg SID). Within one hour the dog's body temperature started to rise from 38,0 C to 40,8 C and it was scratching itself intensively on the neck and face with it's hind legs. It became very restless and disorientated. It's ears became red and swollen and it swallowed with difficulty, so the sedation with midazolame i/v in a dosage 0,2 mg / kg was applied but without success. Body temperature continued to rise to 41,0 °C. The edema of the face and neck intensified and dexamethasone i/v in a dosage 1mg / kg was administered. Intensive cooling with ice cubs rectally and wet towels covering was performed. When dog's body temperature had raised to 41,4 °C carprofen 2 mg / kg i/v was added. Despite all the medicine that the dog had received the swelling of the head, neck and it's left eye were becoming life threatening. Its mental state was poor, it was not responding to anything, just tried to scratch vigorously. Ten hours after administering to the clinic, the dog died. Necropsy and viral tests were performed with suspicion of pseudorabies.

## Results of necropsy and viral tests

Necropsy supported our suspicion of AD by finding diffuse non-suppurative meningoencephalitis and myelitis of the thoracal spinal cord.

The immunofluorescence test (IF), using specific conjugate against rabies virus was negative (data not shown) and therefore excluded rabies as differential diagnosis.

Experimental inoculation of rabbit with material from bitch brain and its death four days post inoculation confirmed the presence of AD-virus. Rabbit itching at the site of contagious material inoculation had been first noted on a second day after inoculation (a.i.). Third day a.i. traumatised skin of about 10 $cm^2$ was noticed. Excoriation of the skin at latero-ventral site of abdomen was well circumscribed and deep - it extended to subcutis. Apathy was noted on second day and nervous symptoms and restlessness were noted on a third day a.i. Rabbit have died on the 4th day a.i.

Isolation of the virus on porcine kidney cell line (PK-15) was successful. Neutralisation of the isolated virus JAN 05/06 with specific positive serum have proved the Aujeszky's disease virus.

## Discussion

Leucocytosis with neutrophilia and lymphopenia were present in the bitch's blood. Since CBC (complete blood count) and biochemistry profile should not show specific changes (3) we presume that leucocytosis, neutrophilia and lymphopenia were not directly connected to the AD virus infection. These blood changes are usually present in acute bacterial infection or severe stress and due to the effects of steroids (6). In our case, they could be explained with severe stress, but also by several disease specific and non-specific pathological lesions found at necropsy like myocarditis, gastritis, enteritis and nephritis.

Higher hematocrite and haemoglobin revealed slight dehydration, which could be the consequence of inability to swallow and loss of liquid through saliva.

Hyperglicemia in our case could appeared due to the seizures and consecutive adrenaline (epinephrine) release, while low potassium levels seems most likely to be a consequence of respiratory alka-

losis (6), which could only be predicted since it was not proved by gas analysis. Urinary bladder has been probably enlarged due to inability to stand upright and urinate, but could be at least partly connected to proliferative and metaplastic lesions found in the bladder mucosa. Mild proteinuria was most probably a consequence of elevated body temperature (6) although multifocal granulomatous nephritis has been found at necropsy. Alkaline urine can support suspicion of respiratory alkalosis and also caused increased urinary excretion of urobilinogen. Swelling of the head and neck became life threatening when complicated with pulmonary oedema and congestion. Pulmonary oedema and congestion were already described complications in AD (5).

From the discussion with the owners it was found out that the bitch did get a raw pork head to eat about a week before the first symptoms appeared. The meat was purchased from the local butcher. The rest of the meat was cooked and used for human consumption. In Slovenia, the last case of AD in a dog was noted more than 20 years ago. Slovenia also tends to acquire AD-free status so thorough epizootic investigation was done about the source of the pork meat in our case. According to our data the pig was imported from Hungary that has already twice acquired status of AD-free country, but has lost it in 2002 (4). Our case shows the possibility of spreading of Aujeszky's disease among European Union members.

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## PRIMER BOLEZNI AUJESZKEGA PRI PSICI

T. Kotnik, S. Suhadolc, P. Juntes, M. Gombač, I. Toplak, P. Hostnik, T. Malovrh, D. Barlič-Maganja, J. Grom

**Povzetek:** Psica Neca, mešanka, sterilizirana, stara 2,5 leta je bila 3. januarja 2006 ob 10.45 uri pripeljana na Kliniko za kirurgijo in male živali Veterinarske fakultete v Ljubljani. Psica je bila prejšnji dan na videz še zdrava, čez noč zaprta v garaži, od zjutraj pa je jokala, ležala je zvita in iz gobca ji je tekla slina. Ni imela nobenih kroničnih bolezni, bila je tudi redno cepljena proti steklini. Po kliničnem pregledu je bila psica zaradi slabega stanja hospitalizirana. Kmalu so se pojavili še znaki intenzivnega praskanja. 10 ur po sprejemu na kliniko je psica kljub intenzivni terapiji poginila. Po ponovnem izpraševanju lastnikov smo izvedeli, da je psica pred 7 dnevi jedla surovo svinjsko meso. Na podlagi anamneze in poteka bolezni je bila postavljena domnevna diagnoza psevdorabiesa. Patomorfološka preiskava je potrdila spremembe, značilne za to bolezen, etiološka diagnoza pa je bila dokazana z biološkim poskusom na kuncu in z izolacijo virusa.

**Ključne besede:** psi, bolezni; pseudorabies – etiologija – diagnostika – patologija; herpesvirus 1, suid – izolacija in čiščenje; biološki poskus; psi

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**Case Report**

# DYSTOCIA IN A FREE-LIVING ROE DEER FEMALE *(CAPREOLUS CAPREOLUS)*

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**Summary:** A post mortem examination was preformed on wild female roe deer *(Capreolus capreolus)* that was found in a state of distress and humanely killed. The hind legs of a fawn were protruding through the doe's rectum, indicating perinatal difficulties. Necropsy revealed two female foetuses, one of which had entered the birth canal in an uncommon position with the hind legs forward. This foetus had perforated the wall of uterine horn and rectum with the hind feet, causing gross intra-abdominal trauma and haemorrhage.

**Key words:** reproduction – pathology; pregnancy, animal; dystocia; uterine rupture – etiology; deer; female

## Introduction

Dystocia is defined as any arduous parturition that is abnormally prolonged or difficult and is one of the most frequent causes of neonatal mortality (1, 2). Abnormal labour entails a vast number of influencing factors that include both maternal and foetal entities. Neonatal mortality in the livestock industry represents a well-known and important cause of reproductive inefficiency and economic loss (1, 2). On the other hand, very little is known about dystocia in free-living ruminants, since most of the current records are of livestock or farmed cervid species. On the territory of Slovenia the population of roe deer is estimated to be between 80.000 to 85.000 animals. However, dystocia that reduces the offspring's viability and/or causes maternal injury is inevitable although perhaps relatively rare in wildlife (3, 4).

## Material and methods

In May 2005, an adult roe deer female (Capreolus capreolus) was submitted to the Veterinary faculty of Ljubljana from a hunting area in lower Carniola in the southeast of Slovenia where the doe was humanely killed. The rather emaciated doe, estimated to be 4-5 years old, was found down in a distressed state and was humanely killed by a gunshot to the brain. The animal had been in labor and both hind legs of a fawn were protruding through the doe's rectum along with herniated intestine. The adult carcass was weighed and measured and a post mortem examination was performed following a standard protocol. The entire genital tract was carefully examined.

## Results and discussion

The haircoat on the lower back and upper parts of doe's hind limbs was covered with foetal fluid and wet. The exterior parts of the genitals and rectum exhibited severe congestion. A section of the doe's small intestines and foetal hind legs protruded from the doe's rectum. Necropsy revealed no evidence of herniation of organs in abdominal cavity as described in the ewe (5). A dorsolateral rupture was observed in the left uterine horn with a 20 cm longitudinal perforation of the uterine wall close to the cervix. Two female foetuses, weighting 1600 g and 1450 g each, were found inside the uterus. Both were proportionately developed and of regular

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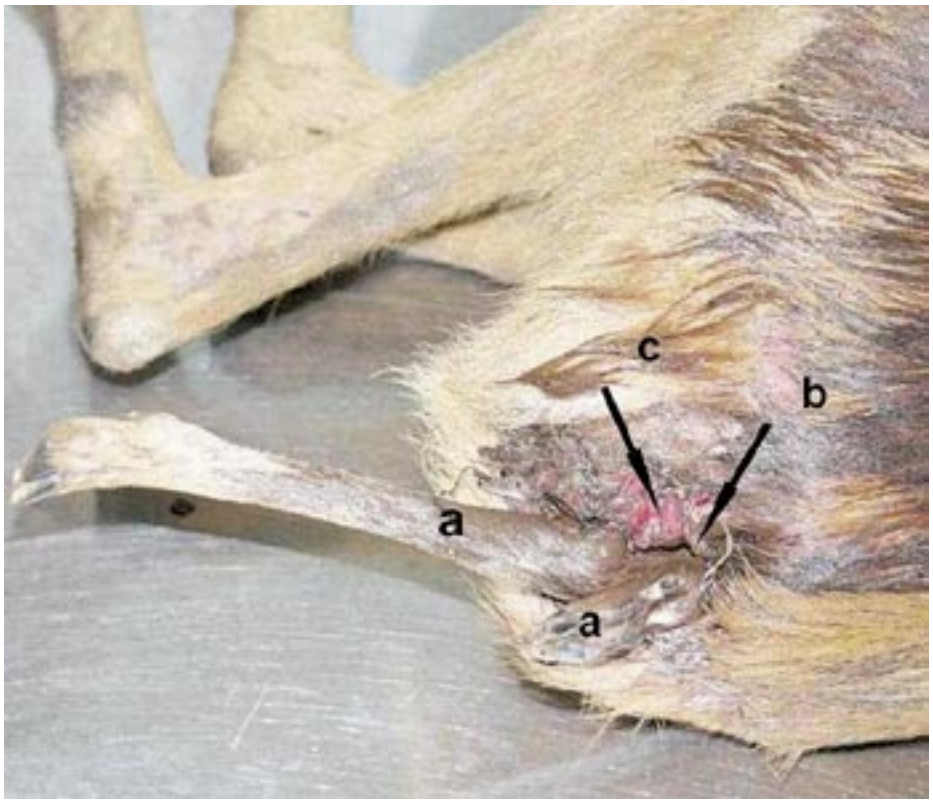


**Figure 1:** Dystocia. Fawn hind legs (a) protruding through doe's rectum (b). Prolapsed, incarcerated and hyperaemic intestine is also evident (c)


**Figure 2:** Fawn hind legs entering the rectum. A haemorrhagic margin surrounds the perforation (b) in left uterine horn (a). Through the rupture in the uterine wall, the foetus hind legs (c) perforated and entered the rectum (d) in a caudodorsal direction. Pelvic musculature also exhibits severe hyperaemia

size with regard to late gestation stage. The fetuses entering the birth canal was in an uncommon posterior position with the hind legs presented first. In this position, the foetus perforated the wall of the uterine horn in an attempt to enter the birth canal. Through the rupture in the uterine wall, the fawn's hind legs also perforated and entered the rectum in a caudodorsal direction (Figure 1). The margin of the rupture in the uterine horn surrounding the perforation was hemorrhagic (Figure 2). Pelvic musculature also exhibited severe hyperaemia (Figure 1). Post-mortem examination did not demonstrate a uterine torsion although one may have been present at some time during labour. Uterine torsion frequently leads to stretching of the uterine ligaments and causes circulatory interruptions in the genital tract. Increased tension of the ligaments provokes abdominal pain which may reflect in tenesmus and organ rupture (5). The uterine cervix was closed and undamaged. There was an extensive intra-abdominal hemorrhage. Necropsy identified no other lesions in any internal organs.

The etiology and pathogenesis of uterine rupture in this doe is not clear. It may have occurred unexpectedly and rapidly at the end of gestation. To the authors' knowledge, this is the first report of such event in a wild Roe deer. Evolutionary, dystocia in wildlife is rare as natural selection provides animals with at most possibilities for survival. Such animals, however, are rarely found.

The incidence of dystocia in livestock is closely linked with animal age. In ewes it appears to be rare at first parturition, but most often occurs in older animals that have already had several pregnancies. Repeated pregnancies with multiple foetuses can predispose to dystocia in older ewes and hinds (4, 5). Some authors reported over 0.5% occurrence of dystocia in deer farms (3). In a recent study of farmed elk stillbirths were mostly related to dystocia (4). Dystocias appears mainly in over-fat hinds (5, 6). Similar findings have been reported in domestic animals such as sheep and cattle (1, 2). Dystocia is also one of the most common causes of perinatal mortality in offspring of domestic animals and farmed deer (3). Some authors agree that good body conditioning, obesity, and lack of exercise in farmed deer contributes to fawning difficulties (3, 5). It was estimated that the first 35 days of life are critical period for a fawn to survive and within that interval the highest rate of mortality occurs (7). A report on Roe deer mortality also indicated that the main cause of death in fawns was stillbirth, followed by starvation/hypothermia, drowning, car accidents and falls (7).

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## PRIMER DISTOCIJE PRI SRNI *(CAPREOLUS CAPREOLUS)*

D. Žele*, A. Bidovec, G. Vengušt

**Povzetek:** Opravljena je bila patoanatomska preiskava pri odstreljeni srni *(Capreolus capreolus)* v agoniji. Zadnji nogi plodu, ki sta viseli iz rektuma, sta nakazovali obporodne nepravilnosti. Ob raztelesbi sta bila najdena dva ploda ženskega spola, prvi je v porodni kanal vstopil v neobičajni legi z zadnjimi nogami naprej. Plod je na svoji poti iz porodnega kanala s parklji zadnjih nog pretrgal steno materničnega roga in rektuma ter povzročil obsežno intraabdominalno krvavitev.

**Ključne besede:** reprodukcija – patologija; brejost; distocija; ruptura maternice – etiologija; srnjad, samica

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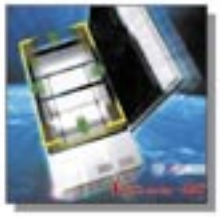

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# SLOVENSKI MEDICINSKI SLOVAR

## TRETJA RAZŠIRJENA IZDAJA


**Uredništvo:** Miroslav Kališnik, *glavni urednik*,
Boris Klun in Dušan Sket, *odgovorna urednika*

**Uredniški odbor:** Mladen Est, Pavle Jezeršek, Miroslav Kališnik, Boris Klun, Marinka Kremžar, Nada Pipan, Alenka Radšel-Medvešček, Dalja Sever-Jurca, Dušan Sket

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Slovar je nastal kot plod četrtstoletnega sodelovanja okrog 150 avtorjev, urednikov, jezikovnih svetovalcev in računalniških strokovnjakov. **Knjižna izdaja 2002** obsega preko 1000 strani ter vsebuje več kot 60.000 biomedicinskih terminov. Namen je dati uporabnikom navodila za pravilno pisanje, izgovarjanje, pregibanje in kratko razlago pomena teh terminov. Med drugim zajema slovenske, poslovenjene in izvirno zapisane grško-latinske termine, generična imena zdravilnih učinkovin, mednarodno sprejete krajšave, osebnosti iz domače in svetovne zgodovine medicine.

Kot plod sodelovanja med Medicinsko fakulteto v Ljubljani in farmacevtsko družbo Lek, Verovškova 57, Ljubljana, je **2003 izšla tudi druga, elektronska izdaja** slovarja. Več informacij je na www.lek.si/slovar. Prednost elektronske izdaje je predvsem preprosto iskanje gesel, tudi njihovih kombinacij, možnost iztiskovanja in vstavljanja lastnih zabeležk. Tiskana verzija pa omogoča udobnejšo čitljivost in preglednost obsežnejših geselskih člankov.

**Tretja, razširjena izdaja bo obsegala okrog 1100 strani ter bo vsebovala za okrog 10 % več gradiva kot druga. Je rezultat dela okrog 60 avtorjev, urednikov in svetovalcev, deloma novih deloma starih. Tretja izdaja pomeni aktualizacijo gradiva. Izid je predviden konec leta 2006.**

Slovar bo prispeval k boljši izobraženosti širokega kroga uporabnikov (biomedicinskih strokovnjakov, študentov, novinarjev in drugih). Slovar je nepogrešljiv za študente in diplomante medicine, stomatologije, veterine, farmacije, biologije in drugih biomedicinskih ved, uporabljajo pa ga tudi drugi delavci v zdravstvu (medicinske sestre, zdravstveni tehniki), prevajalci, novinarji in pacienti.


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Naročam _____ izvod(ov) 3. izdaje Slovenskega medicinskega slovarja.
Cena knjige po izidu bo 30.000 tolarjev (125,19 evra). Do izida pa veljajo prednaročniške cene. Kupci prve knjižne izdaje, ki to dokažejo z iztrganim prvim listom iz knjige, imajo poseben popust pri prednaročilu – 20.000 tolarjev (83,46 evra).
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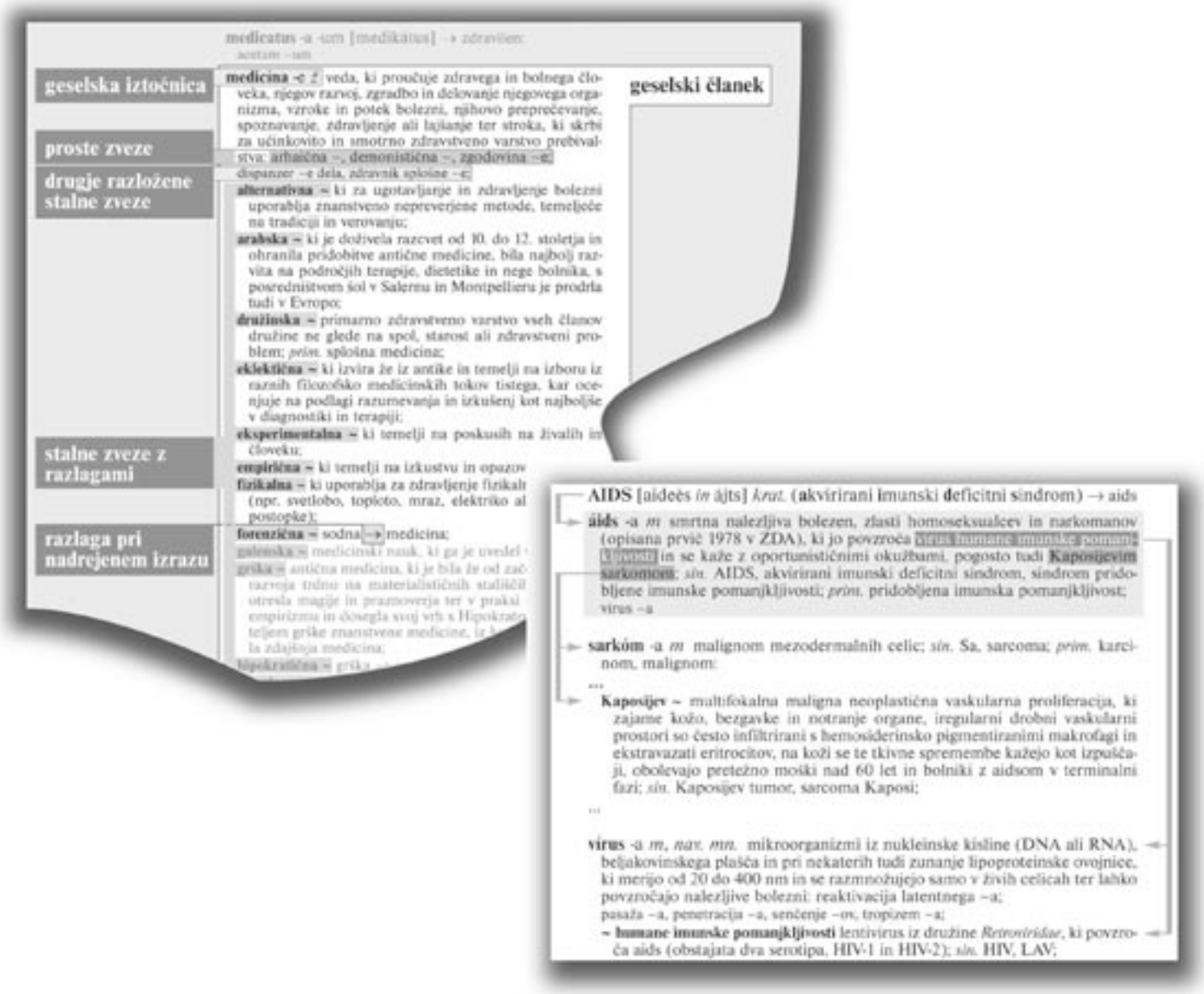


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Any errata should be submitted to the editor-in-chief in good time after publication so that they may be publishedin the next issue.

### Examples of references

**Book:** Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

**Chapterorar ticle in a book:** Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

**Article in a journal or newspaper:** Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

**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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Slovenski veterinarski zbornik (Slovenian Veterinary Research) objavlja izvirne prispevke, ki še niso bili objavljeni oz. poslani v objavo drugam. Za vse navedbe v prispevkih so odgovorni avtorji. Uredniška politika obsega publiciranje znanstvenih člankov, preglednih znanstvenih člankov, strokovnih člankov, povzetkov disertacij in drugih prispevkov, kot so kritične presoje o vsebini razprav, objavljenih v zborniku, kratke znanstvene prispevke, pisma uredniku in drugo. Avtorji pošljejo prispevke na naslov uredništva. Glavni urednik pregleda vse prispevke. Za vse članke je obvezna strokovna recenzija, za katero poskrbi uredništvo.

Prispevki naj bodo napisani v angleškem jeziku, z naslovom, povzetkom in ključnimi besedami tudi v slovenščini. Obsegajo naj največ 12 strani, kar pomeni 27 vrstic na stran s približno 75 znaki v vrstici. Prispevki naj bodo poslani v elektronski obliki v katerem koli urejevalniku besedil za okensko okolje. Zaželjena je uporaba elektronske pošte (slovetres@vf.uni-lj.si) in avtorji naj predlagajo tri možne recenzente. Besedilo naj ima dvojni razmik med vrsticami, pri čemer naj bodo vrstice na levi strani oštevilčene. Besedilo naj bo na levi strani od roba oddaljeno 4 cm.

Naslovna stran prispevkov se začne z naslovom, sledi ime in priimek avtorja. Kadar je avtorjev več, jih ločimo z vejicami. V naslednjih vrsticah je v rubriki Addresses of authors: za dvopičjem treba navesti polno ime in priimek ter naslov(e) avtorja(ev), tj. ustanovo, ulico s hišno številko, pošto in kraj. Vse navedene podatke ločujejo vejice. Sledi vrstica, kjer je treba navesti ime ter elektronski (E-mail:) in poštni naslov ter telefonsko številko (Phone:) odgovornega avtorja.

Sledi besedilo povzetka Summary v obsegu 16 do 20 vrstic (približno 1000 do 1500 znakov). V naslednji rubriki Key words: se za dvopičjem navedejo ključne besede. Posamezne besede ali sklopi besed morajo biti ločeni s podpičjem.

Znanstveni članki in tisti, ki so prikaz lastnih raziskav in dognanj, morajo vsebovati še naslednje obvezne rubrike, s katerimi avtor sam naslovi ustrezne dele besedila v prispevku: Introduction, Material and methods, Results, Discussion in References. Pregledni članki naj vsebujejo uvod, poglavja, ki so glede na vsebino smiselno naslovljena, in literaturo. Podatke o financerjih ali drugih zadevah, pomembnih za prispevek, npr. o tehnični pomoči, avtorji navedejo v rubriki Acknowledgements, ki se uvrsti pred rubriko References. Za rubriko References sledijo spremna besedila k slikam.

Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

Priloge in slike morajo biti poimenovane z besedami, ki jih opredeljujejo, in arabskimi številkami (npr. Table 1:, Figure 1: itn.). Za dvopičjem sledi besedilo oziroma naslov. Vsi navedki (reference), citirani v besedilu, se morajo nanašati na seznam literature. V besedilu jih je treba oštevilčiti po vrstnem redu, po katerem se pojavljajo, z arabskimi številkami v oklepaju. Prvi navedek v besedilu opredeli številko oziroma vrstni red ustreznega vira v seznamu literature. Če se avtor v besedilu ponovno sklicuje na že uporabljeni vir, navede tisto številko, ki jo je vir dobil pri prvem navedku. Citirana so lahko le dela, ki so tiskana ali kako drugače razmnožena in dostopna javnosti. Neobjavljeni podatki, neobjavljena predavanja, osebna sporočila in podobno naj bodo omenjeni v navedkih ali opombah na koncu tiste strani, kjer so navedeni. V seznamu literature so viri urejeni po vrstnem redu. Če je citirani vir napisalo šest ali manj avtorjev, je treba navesti vse; pri sedmih ali več avtorjih se navedejo prvi trije in doda et al.

Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

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**Slov Vet Res 2006; 43 (3)**

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Slov Vet Res 2006; 43 (3): 115-149