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CONTENTS

OLAYEMI, F. O., AZEEZ I. O., OGUNYEMI, A., IGHAGBON, F. O. : Study on erythrocyte values of the nigerian indigenous dog 65

RAHEEM, A. K., FAYEMI, E. O., LEIGH, O. O., AMEEN, S. A.: Selected fertility parameters of west african dwarf bucks experimentally infected with *Trypanosoma congolense* 68

MAĽA, P., VÁCLAVOVÁ, A., BARANOVÁ, M., VIETORIS, V.: Objectivity of percepts of sensory analysis 72

SKURKOVÁ, L., LEDECKÝ, V.: Early diagnosis of canine HIP dysplasia 77

HOLEČKOVÁ, B., DIANOVSKÝ, J., ŠIVIKOVÁ, K.: Cytogenetic effect of pesticides on cultivated peripheral lymphocytes of cattle 83

LUPTÁKOVÁ, L., BÁLENT, P., VALENČÁKOVÁ, A., NOVOTNÝ, F., PETROVOVÁ, E.: Serological detection of antibodies to *Toxoplasma gondii* in animals kept in households 87

HANUŠ, O., JANŮ, L., VYLETĚLOVÁ, M., KUČERA, J.: Research and development of a synthetic quality indicator for raw milk assessment 90

PŘIDALOVÁ, H., JANŠTOVÁ, B., CUPÁKOVÁ, Š., DRAČKOVÁ, M., NAVRÁTILOVÁ, P., VORLOVÁ L.: Somatic Cell Count in Goat Milk 101

JEVINOVÁ, P., MÁRTONOVÁ, M.*, PIPOVÁ, M., POPELKA, P., REGECOVÁ, I., NAGY, J.: Tetracycline resistance of staphylococci isolated from game meat and mechanically deboned poultry meat 106

GUIDE FOR AUTHORS 111

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STUDY ON ERYTHROCYTE VALUES OF THE NIGERIAN INDIGENOUS DOG

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ABSTRACT

The study investigated the influence of sex and age on the erythrocyte values of the Nigerian indigenous dog. No significant (P > 0.05) differences related to sex were observed in the values of red blood cell counts (RBC), packed cell volume (PCV), haemoglobin (Hb) concentration, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV). However, the erythrocyte osmotic fragility was significantly higher in females. When the erythrocyte values of the adults (3 to 4 years old) were compared with those of the young (8 to 10 weeks old), adults showed significantly higher values of RBC (P < 0.01), PCV (P < 0.02) and Hb (P < 0.05) than the young Nigerian indigenous dogs. However, the MCH, MCHC and MCV were similar (P > 0.05) in the two age groups. This study revealed that except for the erythrocyte osmotic fragility, which was higher in females, no sex differences in the erythrocyte values were observed in the Nigerian indigenous dog, however, there were significant age-related differences.

Key words: age; erythrocyte; Nigerian dog; sex

INTRODUCTION

This study is part of ongoing investigations aimed at determination of reference haematological values of the indigenous species of animals in Nigeria and different factors that affect these values. Nigerian indigenous dogs weigh between 15 and 25 kg. The local people use them for hunting in the wild. They also serve as guard dogs and pets in various metropolitan cities of the country. There is an upsurge in the acquisition of this local breed of dogs, which is probably due to their being cheaper to care for than the exotic dog breeds. Also it seems that they are preferred because of their resistance to diseases caused by blood parasites, such as canine babesiosis and trypanosomosis. The Nigerian indigenous dogs are now brought regularly to veterinary clinics for treatment and hence the need to understand the physiology of this local breed. There is presently a dearth of information on the haematology of the Nigerian local dog.

Although there are about 4.5 million Nigerian indigenous dogs (2), only few studies were conducted on the blood profiles. Awah and Nottidge (3) reported the effect of age on serum biochemical parameters of the Nigerian dog. There were some reports on the haematology of the adult Nigerian dog (1, 7, 8, 10, 18). However, none of these studies have documented the effect of sex on the erythrocyte osmotic fragility. Also there is no report on the effect of age on the erythrocyte values of the Nigerian dog. Therefore, in this paper, we report the influence of sex and age on the erythrocyte values of the Nigerian indigenous dog.

MATERIALS AND METHODS

The twenty adult (10 males and 10 females) and five young Nigerian indigenous dogs that were used in this study were apparently healthy. The adults were aged between 3 and 4 years, while the young were 8 to 10 weeks old. They were presented at a Pet Care Veterinary Clinic in Lagos, Lagos State, Nigeria,

for routine clinical assessment of health or for anti-rabies vaccination. The dogs were fed rations of 50 % meat supplemented with rice, beans and some local food. It was ensured that the dogs were not excited before the sampling.

Blood was obtained from the cephalic vein of all the animals between 8:00 and 10:00 a.m., and collected in bottles containing ethylene diamine tetraacetic acid (EDTA) (2 mg.ml^{-1} of blood) as an anticoagulant. Red blood cell (RBC) counts were determined using a haemocytometer. The packed cell volume (PCV) was estimated by the microhaematocrit method and haemoglobin (Hb) concentration by the cyanmethaemoglobin method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described earlier (5). Statistical comparisons were carried out using Student's t-test, with $P < 0.05$ taken as significant.

The osmotic fragility of erythrocytes was determined as described previously (16) using phosphate-buffered sodium chloride solution, pH 7.7, at 29 °C at a concentration of 0.0—0.7 % (Fig. 1). The percentage of haemolysis at each NaCl concentrationl was evaluated by comparison with that of distilled water (0 % NaCl) equal to 100 %.

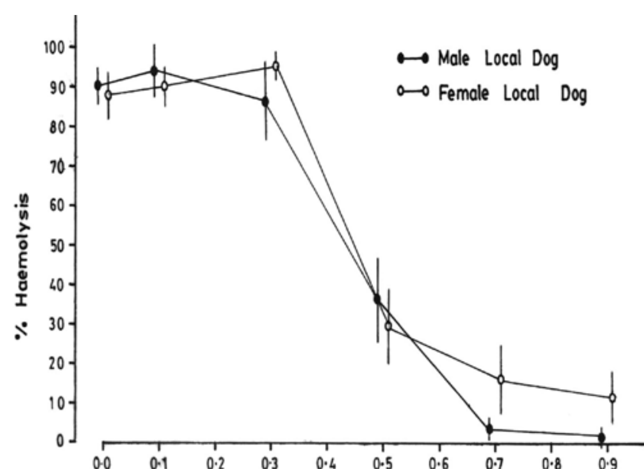

Fig. 1. Erythrocyte osmotic fragility in male and female Nigerian indigenous dogs. Each point represents the mean ± SD for 8 males and 10 females

The values were significantly higher at $P < 0.05$

RESULTS

Table 1 shows the influence of sex on the erythrocyte values of the Nigerian indigenous dogs. No significant differences ($P > 0.05$) were found between the values of RBC, PCV, Hb, MCV, MCH and MCHC in males and females.

Table 2 presents the effect of age on the erythrocyte values of the Nigerian indigenous dog. The adults showed significantly higher RBC ($P < 0.01$), PCV ($P < 0.02$) and Hb concentration ($P < 0.05$) than the young dogs. However, the values of MCV, MCH and MCHC were similar in the two age groups.

Figure 1 shows the influence of sex on the erythrocyte osmotic fragility in the Nigerian local dogs. At 0.7 % and 0.9 % NaCl concentrations, the erythrocytes in the female local dogs were more fragile than those in the males ($P < 0.05$).

DISCUSSION

The erythrocyte count of 6.67×10^{12} per litre was obtained for the Nigerian indigenous dog in the present study (Tab. 1). It was higher than the values of 4.35×0^{12} per litre (7) and 5.13×10^{12} per litre (10) that were obtained for the same breed of dog. It seems the high RBC count of the Nigerian dogs investigated in the present study reflected higher plane of diet that these dogs were given. However the Hb concentration of 136.40 g.l^{-1}, in the present study, was similar to the

Table 1. Erythrocyte values (mean ± SD) of male and female Nigerian indigenous dogs

PARAMETER	MALE (n = 10)	FEMALE (n = 10)
PCV (%)	37.00 ± 3.94	38.80 ± 4.08
Hb conc. (g.l^{-1})	144.2 ± 3.60	150.1 ± 3.60
RBC (x10^{12}.l^{-1})	5.11 ± 0.93	5.00 ± 1.16
MCV (fl)	74.00 ±11.50	80.83 ± 15.93
MCH (pg)	28.64 ± 6.94	30.88 ± 7.36
MCHC (g.dl^{-1})	39.30 ± 10.22	38.51 ± 7.51

The differences were insignificant ($P > 0.05$)

Table 2: Erythrocyte values (mean ± SD) of adult and young Nigerian indigenous dogs

PARAMETERS	ADULT (n = 11)	YOUNG (n = 5)
RBC (X 10^{12}.l^{-1})	6.67 ± 1.20	4.34 ± 1.49*
PCV (%)	43.73 ± 9.57	28.20 ± 9.90**
Hb (g.l^{-1})	136.80 ± 34.10	97.00 ± 27.70***
MCH (pg)	22.02 ± 2.21	22.89 ± 2.93
MCHC (g.dl^{-1})	33.52 ± 0.59	34.42 ± 2.93
MCV (fl)	65.71 ± 6.82	65.44 ± 3.71

Values of young dogs significantly different from those of adult dogs: * — $P < 0.01$; ** — $P < 0.02$ and *** — $P < 0.05$

values of 133.10 g.l^{-1} (7) and 138 g.l^{-1} (10) obtained earlier in this breed of dogs.

The present study showed that the PCV, RBC and Hb values were significantly higher in the adult than in young Nigerian dogs (Tab. 2). This is in agreement with the finding by Bush (4) that the haematological values were lower in the young than the adult dogs. This may be due to the fact that RBC lifespan is shorter in young than in adult dogs. The author also observed that young dogs had less haemoglobin in their RBC than adult dogs. However, our observations contradicted those made by Oluwaniyi *et al.,* (14) who reported that erythrocyte values in young and adult guinea pigs were similar. Our results were also at variance with the findings in the Friesian cattle (17) and White Fulani cattle (9) in which no age-related differences were reported in erythrocyte values.

The present study revealed that the erythrocyte values were similar in male and female Nigerian local dogs (Tab. 1). Ariyibi *et al.* (1) reported similar observations that there were no sex differences in the values of PCV and Hb in the same breed of dogs. It has also been reported that there were no sex-related differences in the erythrocyte values of the White Fulani cattle (11), African giant rat (16) and Nigerian cat (6). However, it was observed that the male White Fulani cattle had lower PCV and Hb values than females (9). Swenson (19) stated that the male androgenic hormone, testosterone, stimulates the production of erythropoietin, which in turn stimulates the process of erythropoiesis and, consequently, results in higher erythrocyte values in male animals. It seems that the lack of sexual dimorphism in the Nigerian indigenous dogs observed in the present study was related to the fact that these dogs were bled outside their breeding season when the hormonal influence on blood values was minimal.

This is the first time that the erythrocyte osmotic fragility would be determined in the Nigerian local dog. The osmotic fragility of erythrocytes was greater in female than in the male Nigerian dog (Fig. 1). The observations made in the present study resembled those in the African giant rat (*Cricetomys gambianus*, Waterhouse) (16) in which the female exhibited greater erythrocyte osmotic fragility than the male. The observations in the present study were however at variance to those made in the White Fulani cattle (11) and N'dama cattle (12) in which the male was reported to have greater erythrocyte osmotic fragility than the female.

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SELECTED FERTILITY PARAMETERS OF WEST AFRICAN DWARF BUCKS EXPERIMENTALLY INFECTED WITH *Trypanosoma congolense*

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ABSTRACT

Selected fertility of West African Dwarf (WAD) bucks experimentally infected with *Trypanosoma congolenese* was investigated in a study that lasted 16 weeks. These parameters were libido, scrotal length and circumference as well as semen characteristics — volume, colour, mass activity, motility, live/dead ratio and sperm concentration. The animals served as their own control, the data obtained at the pre-infection period were regarded as the control with which the post-infection data were statistically compared.

There was a significant reduction in the libido score from the control mean value of 9.25 ± 0.96 to a minimum value of 6.00 ± 1.33 at the 10th week post-infection ($P < 0.05$). The changes in the scrotal length and circumference before and after infection were minimal and insignificant ($P > 0.05$). The mean percentage motility and live/dead ratio before infection were 92.06 ± 3.83 % and 96.87 ± 1.5 % respectively. These were significantly reduced to minimum values of 58.75 ± 6.3 % and 60.0 ± 5.00 % at the post-infection period, respectively ($P < 0.05$). The concentration of the spermatozoa declined significantly from the pre-infection mean of $2.19 \pm 1.87 . 10^9$ ml^{-1} to a minimum of $1.28 \pm 0.09 . 10^9$ ml^{-1} ($P < 0.05$). The mean per cent of abnormal spermatozoa increased significantly from the baseline value of 4.92 ± 2.45 to 37.13 ± 8.12 by the week 10 post-infection. The results show that *Trypanosoma congolense* caused poor semen quality, and reduced libido. Therefore, infertility is very imminent in *Trypanosoma congolense* infected bucks with accompanied economic loss, especially in tsetse-infected areas of the Sub-Saharan Africa. Therefore, screening for trypanosomiasis in the course of investigation of infertility in farm animals in these areas should be a welcome initiative.

Key words: fertility; libido; semen; *Trypanosoma congolense;* West African Dwarf bucks

INTRODUCTION

Trypanosomiasis has been labelled as one of the major constraints to livestock industry and food security in the Sub-Saharan Africa (26) with an estimated direct loss in meat and milk yield as well as cost of disease control amounting to the level of $600 million to $1.2 billion annually on the global scale (6). Despite the socio-economic importance of goat in developing countries (7, 9, 19), this animal species has received little attention generally (17) and in trypanosomiasis studies specifically (4). The initial conception that WAD bucks are trypanotolerant (14) contributed to this apathy, while evidence confirming natural infection with reproductive

dysfunction abound (5, 20). Moreover, the tendency of these small ruminants to serve as reservoir hosts in tsetse-infested areas (12) has emphasized the need to intensify investigations of the disease in them. The paucity of knowledge as regards reproductive implications of *Trypanosoma congolense* on fertility parameters of WAD bucks stimulated this study.

MATERIALS AND METHOD

Five apparently healthy matured bucks and one doe were sourced from different locations within the Ibadan metropolis. The study was carried out at the Department of veterinary surgery and reproduction, University of Ibadan. The animals were first screened for trypanosomes using Buffy Coat Method (15). The study spanned a period of 16 weeks. The first 2 weeks were used to stabilize and acclimatize the animals to the new environment. The 3rd to 6th week was the pre-infection period. Infection with Gboko strain of *Trypanosoma congolense* was done at the end of the 6th week with 2 ml of diluted blood containing approximately $10 . 10^6$ trypanosomes via the intraperitoneal route. The parasites were originally obtained from a goat with clinical trypanosomiasis at Gboko, Benue State in 1988. The 7th to 16th week was termed post-infection period. The animals served as their own control — the data obtained at the pre-infection period, regarded as the control, were compared with the post-infection data. All data were taken between the 07.00 and 09.00 hour before feeding. The clinical parameters monitored were rectal temperature, body weight, parasiataemia level and full haematology with emphasis on Packed Cell Volume (PCV).

Blood samples were collected from the jugular vein. The blood was used to estimate the PCV using a standard micro-haematocrit centrifugation technique (23) and parasitaemia according to the method described by (15). The scrotal length and circumference were measured using a flexible measuring tape (16).

Libido score was obtained using the method described for bulls by Osborne *et al.* (18). Briefly, this method involved having an ovariectomised doe at hand. The ovariectomy in the doe was done using the method described for Caesarean section in doe by Jackson (8). The doe was then treated intramuscularly with 5 mg progesterone injection (Gestroll^R, Jinling Pharmaceutical Group, China) for three consecutive days followed by 10 mg of oestrogen (Estradiol Cypionate ECP^R, Upjohn, USA) injection on day 4. The doe exhibited behavioural oestrus 12—18 hours after the last injection and could be used for 4 consecutive days simply by administering it 5 mg estrogen the previous evening. The procedure was a depletion time and involved introduction of the female to the male individually for a period of 5 minutes. The scoring was done using the libido scoring system (Tab. 1). Each buck was tested twice and the best score was recorded.

Semen was collected once a week by the electroejaculation method and analysed by a standard technique (27). The means and standard deviations of the measurements were calculated and all variables were statistically analysed using analysis of variance at 5% level of significance (25).

Table 1 . Libido Scoring System

0	Buck shows no sexual interest
1	Sexual interest shown only once (e.g. sniffing of the perineal region)
2	Positive active sexual interest in the female on more than one occasion
3	Active pursuit of the female with persistent sexual interest
4	One mount or mounting attempt with no service
5	Two mounts or mounting attempts with no service
6	More than two mounts or mounting attempts with no service
7	One service followed by no further sexual interest
8	One service followed by sexual interest including mounts or mounting attempts
9	Two services followed by sexual interest including mount and mounting attempts
10	Two services followed by sexual interest including mounts, mounting attempts and further service

RESULTS

The clinical symptoms, even though not pathognomonic, recorded in this study were dullness, starry hair coat, weight loss, hyperthermia and pale mucous membranes with enlarged superficial lymph nodes. The summary of the clinical parameters are presented in Table 2. There was an insignificant reduction (13 %) in the live body weight of infected animals at the end of the study ($P > 0.05$). The parasite was detectable in the peripheral circulation within week 1 post-infection ($4.34 . 10^7$ trypanosomes/ml) and the parasitaemia profile was cyclical in pattern, reaching the maximum of $4.36 . 10^7$ trypanosomes/ml at week 1 post-infection and minimum of $1.43 . 10^7$ trypanosomes/ml at week 7 post-infection. The difference between the mean values at pre-infection and post-infection period was statistically significant ($P < 0.05$). The rectal temperature changed significantly from the normal pre-infection value (39.3 ± 0.53 °C) and remained above the normal throughout the post-infection period with peak value of 41.9 ± 0.58 °C observed at week 10 post-infection

There was a significant reduction ($P < 0.05$) in the libido score from the control mean value of 9.25 ± 0.96 to a minimum value of 6.0 ± 1.33, observed at week 10 post-infection. The summary of the semen characteristics is presented in Table 3. The semen colour before the infection was usually creamy white, changed gradually to milky white and tended towards watery at the end of the study. The change in the mean scrotal length and circumference before and after the infection was minimal

and insignificant so was the reduction in semen volume. The mean percent motility and percent live/dead ratio at the control level (92.06 ± 3.83 % and 96.87 ± 1.5 %, resp.) declined significantly ($P < 0.01$) to minimum values of 58.75 ± 6.3 % and 60.0 ± 5.0 %, respectively. The sperm concentration before the infection was $2.19 \pm 1.87 \cdot 10^9 ml^{-1}$ and decreased significantly to a minimum mean value of $1.28 \pm 0.09 \cdot 10^9 ml^{-1}$. The mean percentage abnormal spermatozoa prior to the infection was 4.92 ± 2.45 % and increased significantly to a maximum value of 37.13 ± 8.12 % ($P < 0.05$).

Table 2. Changes in clinical parameters and libido score (mean ± SD) of West African Dwarf bucks following experimental infection with *Trypanosoma congolense*

Week post infection	0	1	4	7	10
Body weight (kg)	9.58 ± 3.60	9.33 ± 2.49	9.26 ± 2.29	9.15 ± 2.28	8.33 ± 2.21
Rectal Temp. (°C)	39.3 ± 0.53	40.6 ± 1.34	40.0 ± 0.76	40.4 ± 0.33	41.9 ± 0.58
PCV (%)	27.0 ± 4.76	21.2 ± 2.86	15.0 ± 6.0	18.62 ± 3.28	18.10 ± 4.26
Parasitaemia ($\times 10^7.ml^{-1}$)	0	4.36 ± 1.36	2.38 ± 0.76	1.43 ± 0.50	2.75 ± 2.0
Libido score	9.25 ± 0.96	9.00 ± 0.82	6.0 ± 0.82	6.5 ± 2.38	6.0 ± 1.33

Table 3. Spermiogram (mean ± SD) of West African Dwarf bucks following experimental infection with *Trypanosoma congolense*

Week post infection	0	1	4	7	10
Volume (ml)	0.41 ± 0.13	0.35 ± 0.1	0.25 ± 0.06	0.2 ± 0.04	0.13 ± 0.05
Motility (%)	92.06 ± 3.83	90.0 ± 4.08	82.5 ± 2.89	58.75 ± 6.3	62.5 ± 12.58
Live/Dead ratio (%)	96.87 ± 1.5	95.25 ± 3.78	90.0 ± 4.08	60.0 ± 5.0	65.0 ± 0.58
Sperm Conc. ($\times 10^9$.ml)	2.19 ± 1.87	2.02 ± 1.08	1.64 ± 1.91	1.53 ± 0.91	1.28 ± 0.09
Abnormal spermatozoa (%)	4.92 ± 2.45	18.25 ± 3.84	23.14 ± 5.62	33.35 ± 2.86	37.13 ± 8.12

DISCUSSION

Clinical symptoms of trypanosomiasis observed in the study have been described in similar studies (3, 22). No death was recorded despite the decline in general body condition of the infected animals in contrast to the findings of Anosa (3). The high survival rate may be attributed to the trypanotolerance of WAD goats and good management practice that reduced the risk of concurrent infections. The cyclical parasitaemia observed is in agreement with the findings of Anosa *et al.* (5) and Saror (22). The reduction in the libido score was in agreement with the report of Sekoni *et al.* (24) in bulls. The mechanism of *Trypanosoma congolense* causing decline in libido may be due to decline in testosterone. Testosterone plays an important role in optimal functioning of the testis and initiation of the sex drive (11). Though testosterone assay was not done in this study, there were reports of reduced testosterone level associated with trypanosomiasis in bulls (1), rams (2) and goats (28).

The changes in the semen characteristics agreed with the findings of other authors involved in similar studies (3, 22). The deterioration in semen quality at trypanosome infection was attributed to testicular degeneration. the pathogenesis of which involves thrombosis of testicular vessels leading to ischaemic necrosis (5), thermal degeneration experienced especially at the peak of hyperthermia (20) and anoxia (10). Although the sperm concentration complied with the minimum requirements for fertile bucks (13), other semen characteristics, particularly sperm morphological features, deviated from acceptable values. Sperm morphology is an essential parameter that reflects the degree of normality and maturity of the sperm population in the ejaculate and correlates with fertility (13). Hence, infertility is imminent in *Trpanosoma congolense* infected animals. We conclude that screening for trypanosomiasis in the course of investigating infertility in farm animals should be a welcome initiative, especially in tsetse-infested areas.

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OBJECTIVITY OF PERCEPTS OF SENSORY ANALYSIS

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ABSTRACT

The experiment consisted of a colour word reading and ink colour naming tests based on the original Stroop's tests. There were a total of 31 mistakes in the optical illusions made by the participants. The most mistakes occurred when defining brown colour where the incidence ratio was 0.258. The probability of such a phenomenon was 9%. The least mistakes occurred when defining pink colour, with the incidence ratio of 0.0645. To sum up, according to our results of naming basic colours we can say that students were wrong when defining blue (0.258) and black colour (0.258). The most mistakes were made at the end of optical illusion effect when defining pink colour (0.37) which was confused with red colour. Probability of such a phenomenon was 9%. The lowest ratio was reached when defining red, white and yellow colour 0.161.

Key words: colour; Stroop effect; tree diagram

INTRODUCTION

Human ability to carry out mental tasks is interfered with by many objective and subjective factors (1, 2, 3). We can perform some activities concurrently, for example driving a car and listening to the radio, without mutual interference. On the other hand, there are cognitive tasks which affect each other although they do not seem related at the first sight.

The phenomenon of a mutual affect of cognitive processes is called interference. The interference effect suggests you are not always in complete control of what you pay attention to. One of the most known examples of the interference is Stroop effect (4) which illustrates the interference among cognitive processes of reading and recognizing colours.

Stroop showed that the human capability to name ink colour of the text depends on what is written. Stroop studied interference tasks to the tasks oriented to the colour word reading and colour naming. In the first experiment he compared reading time necessary to read a colour names printed in black. He also compared a time necessary for recognizing a colour of the word that is printed in a different colour than its meaning (word RED printed in green colour – the correct answer is green) and also the recognition of the colour of a solid colour rectangle. Since then, more than 700 studies have been carried out with the aim to justify Stroop effect – disability to avoid reading the word. New justifications agree with the parallel distributed models.

Different tasks develop different processing pathways and practice as well as biological wiring, create different pathway strengths. Consequently it is strength, not speed that is basic. Additionally, the degree of automatic is a function of the strength of each pathway. What this means for the Stroop's task is that if two pathways are active simultaneously and the pathway that leads to the response is stronger (naming words), no interference occurs. However, if two pathways are active simultaneously and the pathway that leads to the response is weaker (naming the colour of the word), interference results.

We can conclude that the interference among cognitive processes of reading a word and colour naming occurs already in the phase, when the subject is recognizing a colour, not when he is naming a colour. It demonstrates the difference in data processing between right and left hemispheres.

MATERIAL AND METHODS

The method of Stroop effect test is based on a principle of participant concentration who is given a variety of modes of stimulus presentation accidentally chosen from the computer program. The importance is given to a colour of a word that is being read and not of a content (meaning) of a word that is naming a colour. It is possible to create hundreds of combinations from 49 sorts of colour. Concentration is based on the evaluation of the mistakes made and time limits.

Tree diagrams represent a group of rules that lead to precise value or a placement into a correct group. The tree diagram starts with one item that branches into two or more, each of them branching into two or more, and so on. It looks like a tree with trunk and multiple branches.

The main tree mode is a decision node, defines a question or a test that has to be evaluated. Possible answers to the questions divide a tree into branches. Number of potential answers equals to a number of branches created. Number of branches depends on the algorithm applied. Each branch then leads to another decision to possible outcomes. Each decision node evaluates input data that were given and defined at the beginning of the problem. The probability of any outcome in the sample space is the product (multiply) of all possibilities along the path that represents that outcome on the tree diagram.

These tree diagrams are then used to state a decision that needs to be made, predictions or rating. Some algorithms can be applied to create a tree diagram, for example CHAID (Chi-squared Automatic Interaction detection), CART (Classification and Regression Trees), Quest a C5.0. (5). Developing the tree diagram helps you move your thinking step by step from generalities to specifics.

The study involved 18 students in the 3rd year of Food Hygiene study programme at the University of Veterinary Medicine in Košice with average age of 21, 12 women and 6 men, and 32 students in the 6th year of Food Hygiene study programme from the same university, with average age of 24, 21 women and 11 men. Nobody suffered from colour blindness or any other visual problem. We created 11 optical illusion models. Each of the participants was given one accidentally chosen test. His/her task was to name a colour of the word, not to read a word. We observed mistakes that were made when naming a colour and we measured a time necessary to complete the task. The results were registered in a table and were evaluated statistically using a decision tree. The objective of the study was to find out how difficult it is to control an automatic process of reading and inability to avoid reading the word and also to evaluate the concentration of the participants.

RESULTS AND DISCUSSION

From the results shown in Tables 1 and 2 we can say, that when the words were printed in a colour differing from the colour expressed by the words semantic meaning, the participants use to make mistakes, but the result was not so strong. This interference is thought to have been caused by the automation of reading as the mind automatically determines the semantic meaning of a word, and then must override this first impression with the identification of the colour of the word, a process which is not automatic.

In Table 1 we present reactions of 18 students in the 3rd year of Food Hygiene study programme at the University of Veterinary Medicine in Košice. Analysis of

Table 1. Reactions of 18 students in the 3rd year of Food Hygiene study programme at the University of Veterinary Medicine in Košice in the test on the Stroop effect

Optical illusion No.	Time /s	Number of mistakes	Position of mistaken word in optical illusion	Correct colour	Incorrect colour
1	78	2	14	brown	black
			16	brown	white
2	41	0			
3	43	0			
4	50	1	39	red	brown
5	71	1	37	black	purple
6	55	2	21	pink	yellow
			48	pink	grey
7	58	1	8	orange	white
8	45	1	17	yellow	blue
9	63	4	3	brown	grey
			7	white	blue
			31	purple	green
			46	purple	Pink
1	42	2	15	black	brown
			45	white	yellow
2	48	0			
3	50	2	11	purple	red
			47	pink	grey
4	43	1	4	yellow	green
5	50	1	42	orange	purple
6	62	2	28	pink	blue
			36	white	blue
7	45	1	26	red	blue
8	50	0			
9	65	2	12	white	grey
			36	pink	purple
Total	**53.28**	**1.27**			

Table 2. Reactions of students in the 6th year of Food Hygiene study programme in the test on the Stroop effect

Optical illusion No.	Time /s	Number of mistakes	Position of mistaken word in optical illusion	Correct colour	Incorrect colour
1	52	1	25	yellow	orange
2	43	4	26	black	brown
			28	white	green
			47	orange	brown
			49	orange	black
3	44	1	42	green	grey
4	54	0			
5	50	0			
6	51	0			
7	61	3	17	brown	black
			19	brown	green
			30	brown	red
8	43	0			
9	59	1	18	brown	blue
10	61	3	19	yellow	white
			28	blue	blue
			34	white	white
11	66	2	10	black	red
			14	pink	black
12	48	0			
13	38	1	23	purple	white
14	55	3	22	pink	orange
			30	white	red
			35	brown	orange
15	39	1		white	orange
16	52	3	9	pink	white
			15	orange	yellow
			44	pink	black
1	59	1	24	green	green
2	36	1	43	orange	grey
3	70	0			
4	56	1	11	green	purple
5	34	1	41	blue	brown
6	30	0			
7	46	2	21	pink	red
			45	yellow	brown
8	35	0			
9	53	2	2	black	brown
			18	brown	blue
10	43	2	15	brown	purple
			30	grey	black
11	37	1	39	grey	black
12	35	0			
13	51	2	34	purple	yellow
			45	purple	pink
14	46	1	25	white	yellow
15	65	1	36	blue	grey
16	58	1	22	yellow	purple
Total	**47.48**	**1.21**			

the tests indicated that most mistakes were made in the second part of the experiment, when the concentration decreased. The most confusing colour was blue (5 times) and purple (3 times) and, on the contrary, the least confusing colour was black (1 time), red (1 time), pink (1time) and yellow (1 time). The average time spent was 53.2 seconds and the incidence ratio was 1.27.

Table 2 shows reactions of 32 students in the 6th year of Food Hygiene study programme at the same university. The most mistakes were made when defining brown (6 times) and orange colour (4 times), the least mistakes were made with pink colour (1 time). The average time spent was 47.48 seconds and average incidence ratio was 1.21.

Fig. 1 shows the results of a Stroop effect for mixed colours. There were 31 mistakes made related to optical illusions. Then we calculated the incidence ratio for individual colours. From this incidence ratio one can calculate the probability of a mutual confusion of the colours.

Most of the mistakes were made when defining brown colour and the incidence ratio for this colour was the highest (0.258). It was the most confusing colour in the first part of the optical illusion with the majority of students (0.375) while the correct answer was black colour. The probability of such a phenomenon was 9 %. The same number of mistakes was made when defining grey and purple colour (0.935), mostly in the final part of the optical illusion. The least confusing colour was pink, with the incidence ratio of 0.0645.

Fig. 2 shows the results of a Stroop effect for foundation colours. Most of the mistakes were made when

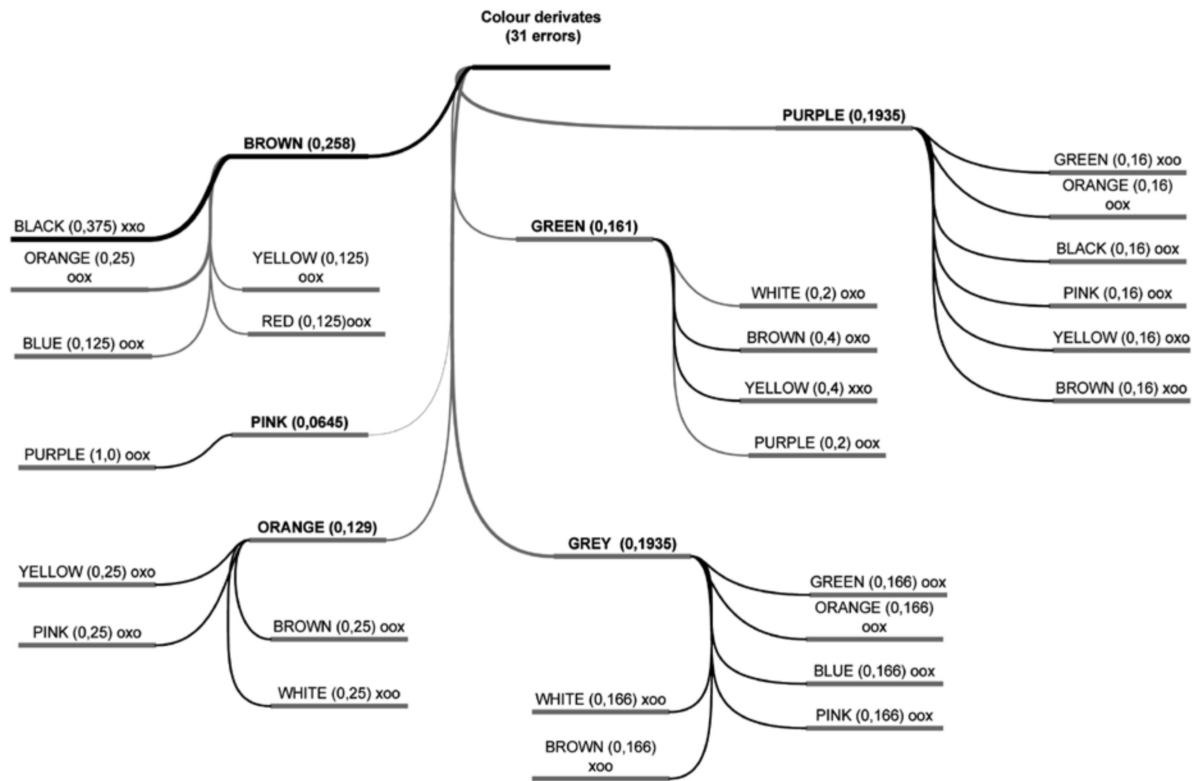

Fig. 1. Decisive tree diagram of Stroop effect results for intermingled colours; 31 analyzed mistakes x – mistake position in optical illusion (1st part: word 1–15 of optical illusion, 2nd part: word 16–30 of optical illusion, 3rd part: word 31–49 of optical illusion)

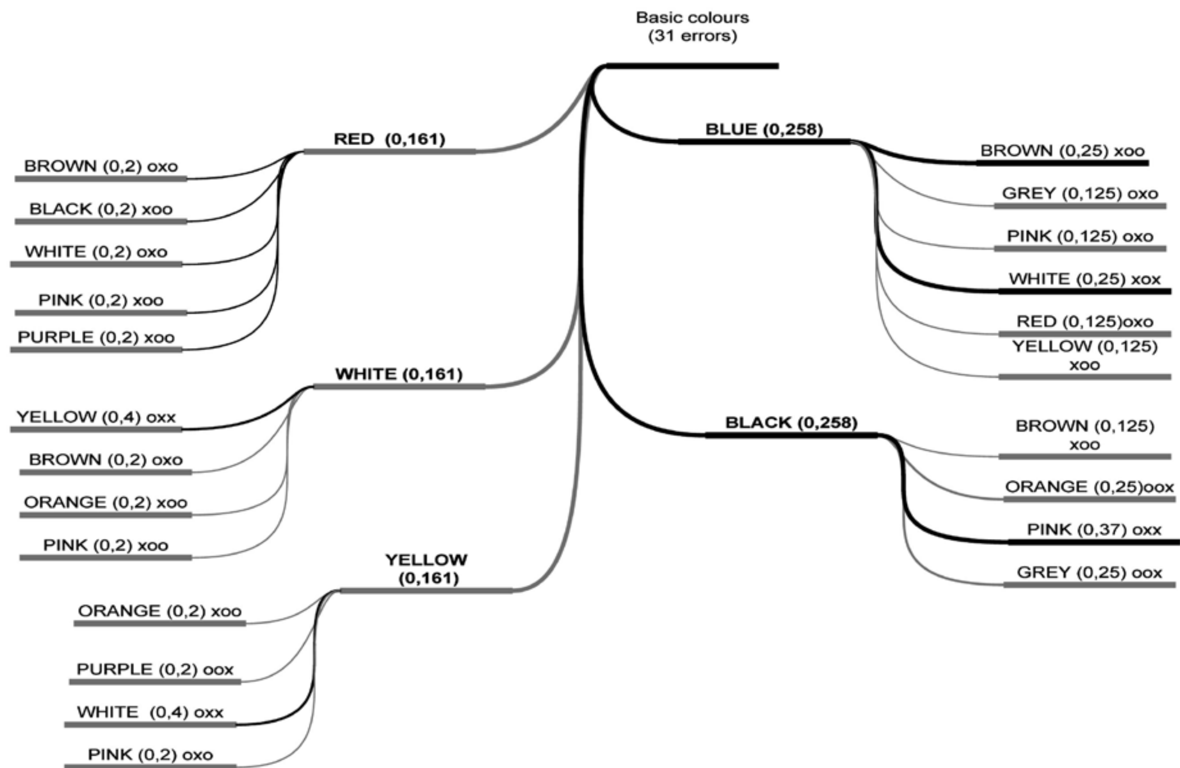

Fig. 2. Decisive tree diagram of Stroop effect results for foundation colours; 31 analyzed mistakes x – mistake position in optical illusion (1st part: word 1–15 of optical illusion, 2nd part: word 16–30 of optical illusion, 3rd part: word 31–49 of optical illusion)

defining blue (0.258) and black colours (0.258). The most confusing colours in the first part of the optical illusion were white and brown (0.25) and both were confused with blue colour. The probability of such a phenomenon was 6 %. At the end of the optical illusion the most confusing colour was pink (0.37) which was confused with black colour (0.258). The probability of such a phenomenon was 9 %.

The least confusing colours were red, white and yellow (0.161). To sum up, with a convenient combination of colours and their incidence ratio, we can make out optical illusions with the most difficult defining colours. This can be used for educational purposes of the evaluators.

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EARLY DIAGNOSIS OF CANINE HIP DYSPLASIA

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ABSTRACT

We investigated effectiveness and potential use of early diagnosis of hip joint dysplasia in German Shepherd dogs by X-ray examination comparing the conventional extension position I with compression-distraction method. In addition to that we also used Ortolani palpation technique and X-ray examination of the hip joint in order to obtain view of its dorsal acetabular rim (DAR) and dorsal acetabular rim angle (DARA) with respect to the femoral head. Examinations were carried out first on dogs 4–6 months old and repeatedly when they reached age of 12 months. We examined altogether 25 German Shepherd dogs but only 10 of them were used for evaluation because for the remaining ones we lacked one or more relevant measurements. Even if the evaluations included all of them, the results were not influenced significantly.

When evaluating the early diagnosis, we used the Norberg angle (NA), distraction index (DI) and evaluated every hip joint by assigning to it a hip dysplasia score according to the FCI (Fédération Cynologique Internationale) dysplasia scheme. When comparing the values by means of Pearson correlation coefficient we detected significant correlation between DI12/FCI12 (distraction index at the age of 12 months/FCI score at the age of 12 months) and DI4/FCI12 (distraction index at the age of 4 months in comparison with FCI evaluation at the age of 12 months) (0.86 and 0.78, resp.). When presenting the early prognosis on the basis of DI and DARA values at the age of 4 months, we observed agreement with the final diagnosis made at the age of 12 months on the basis of NA values and FCI score in 80 % of evaluated dogs.

Key words: *dorsal acetabular rim;* hip dysplasia; Ortolani sign; PennHIP

INTRODUCTION

Hip dysplasia is a developmental orthopaedic disease with polygenic heritability manifested by decreased congruence between femoral head and *acetabulum* resulting in degenerative damage to the joint (7). In contrast with similar disease in humans, it develops in dogs only after birth and can be diagnosed after reaching adulthood (from 12 months of age). Because the possibility of affecting dysplasia and the subsequent development of secondary hip joint arthrosis in adulthood is limited there has been an effort to find new diagnostic procedure usable at earlier age and enabling early therapy. This is based on the knowledge that dysplasia develops on the basis of incongruence of articular facets and excessive joint laxity. This condition can be detected by palpation or X-ray examination which can be carried out starting from the age of 16 weeks.

Of the palpation methods used in human medicine Ortolani (8) and Barlow (3) tests were employed in dogs. In 1968 Bardens described palpation method used specifically for canine hip joints (2).

New X-ray positions for evaluation of passive laxity were developed at Pennsylvania University and the compression-distraction method, called PennHIP (Pennsylvania Hip Improvement Program) was described by Smith *et al.* (10). The outcome of this method is a numeric expression of joint laxity in the form of indexes, the distraction index (DI) and compression index (CI), acquiring values between 0 and 1. Joint

Fig. 1. Positioning for distraction radiological view

Fig. 2. Positioning for compression radiological view

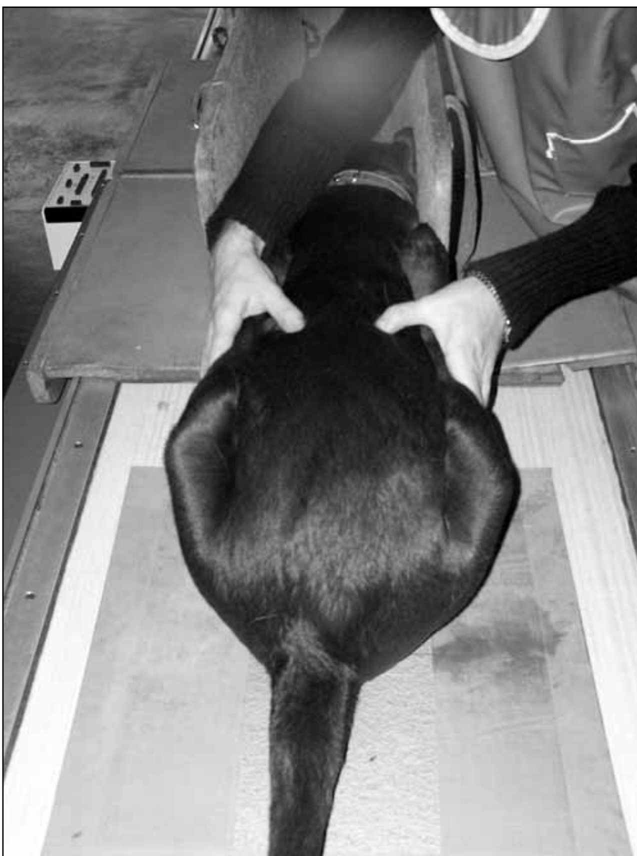

Fig. 3. Positioning for dorsal acetabular rim radiological view

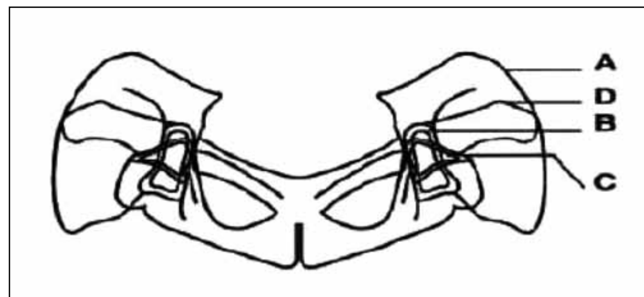

Fig. 4. Superimposition of pelvic structures (dorsal acetabular rim view)
***A – ala ossis ilii, B – corpus ossis ilii,* C – *dorsal acetabular rim*, D – *tuber ossis ischii* (Slocum *et* Devine, 1990)**

laxity is considered a predisposition to later development of canine hip dysplasia (CHD).

Another possibility is the use of a pelvic position affording the view of the dorsal acetabular rim (9) that is used to determine the values of tangent angle and dorsal acetabular rim angle.

The aim of our study was to evaluate the usability of the early examination by the compression-distraction method modified by Vezzoni (14), measurement of dorsal acetabular rim and Ortolani palpation test for making prognosis of the development of hip joint and its laxity in a growing puppy and indication of early surgical therapy in comparison with conventional radiographic methods of canine hip dysplasia diagnosis, carried out at the age of 12 months.

MATERIAL AND METHODS

We carried out X-ray examination by 3 different methods in 10 German Shepherd dogs (i. e. 20 hip joints), and 5 of them (i. e. 10 hip joints) underwent also palpation Ortolani test (dogs were positioned in lateral recumbency and pressure was applied on the *femur* in dorsal direction and, subsequently, the limbs were abducted and the examiner watched for presence of an audible “clunk”). Examinations were done in 4 month old dogs and repeatedly in the same dogs at the age of 12 months.

The X-ray examination was done under sedation (butorphanol – 0.2 mg.kg^{-1} i. m., xylazin – 1 mg.kg^{-1} i. m., with atropine premedication – 0.03 mg.kg^{-1}i. m.). The dogs were examined using an apparatus GIERTH HF 200-H. The blue programme X-ray film, type A – 400, dimensions 30 × 40 cm (24 × 30 cm) was used.

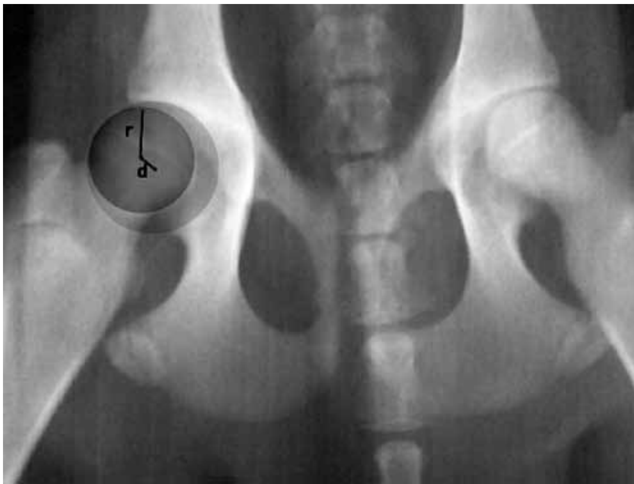

Fig. 5. Measurement of distraction angle (distraction radiograph)

Fig. 6. Measurement of dorsal acetabular rim angle (DARA = 8°)

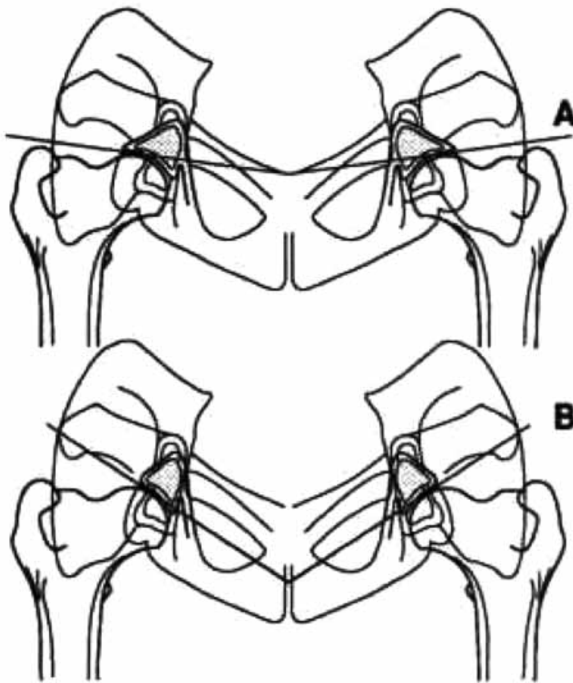

Fig. 7. Tangential angle
A – 165–180° = non-dysplastic hip joint, B – less than 165° = dysplastic hip joint (Slocum *et* Devine, 1990)

Positions at X-ray examination

Method I. – Standard ventrodorsal view: The dog was first placed into ventrodorsal position with extended limbs which allowed us to obtain the conventional hip extension view.

Method II. – Compression and distraction views: The dog was positioned in dorsal recumbency with limb extended backwards, with the hip distraction ensured by means of an Italian distractor FSA (Fondazione Salute Animale). We tried to ensure maximal extension of heads from the *acetabulum* not only by means of the distractor but also by positioning of limbs, with *femur* at a 90–120° angle with respect to the table plane and *tibia* parallel to the table (Fig. 1).

Compression radiograph was obtained in ventrodorsal recumbency with extended pelvic limbs and an adjustable textile band fitted and tightened to the hip joint area. This ensured better engagement of femoral heads into the *acetabulum* (Fig. 2).

Method III. – Dorsal acetabular rim view: Finally the dog was positioned in sternal recumbency and the hind limbs were tractioned cranially and close to the body, with femur at 90° angle with respect to *tibia* (Fig. 3).

The radiograph must show visible pelvic structures (Fig. 4).

Evaluation of radiographs

Method I.: In the standard ventrodorsal position we evaluated 6 parameters important for canine hip dysplasia classification: the Norberg angle, ratio of the femur head and dorsal edge of the *acetabulum*, characteristics of the cranio-lateral edge of the *acetabulum*, characteristics of the subchondral bone, characteristics of the head of femur and its neck and presence of Morgan's line. When assigning the scores we used the method by Flückiger (5), evaluating every joint separately and assigning it points ranging between 0–5. The points were added up and the final degree of dysplasia (A, B, C, D, E) was determined according to the international FCI scheme.

Method II.: The distraction radiograph was used to determine distraction index by dividing the distance between the centre of femoral head and the centre of the *acetabulum* by the radius of the femoral head (DI = d/r; where d is the distance between respective centres and r is the radius of the femoral head) (Fig. 5). The DI can acquire values between 0 and 1.

The compression radiograph was evaluated only visually, assessing the engagement of the femoral head in the *acetabulum*, i.e. congruence of the joint.

Method III.: When evaluating the dorsal acetabular rim views, we measured the angle between the dorsal acetabular rim (DAR) and the tangential angle. Dorsal acetabular rim angle is obtained in such a way that one line is traced along

Tab. 1. Prediction of the development of canine hip dysplasia (CHD) according to the early diagnosis results (4–6 months old dogs) (Vezzoni, 2004)

Prognosis	Ortolani test	FHC/DAE*	Dist raction index	DARA**
normal joints	negative	FHC medial to DAE	0.2–0.4	< 6°
normal – mild CHD	positive	FHC on DAE	0.3–0.5	< 7.5°
mild – moderate CHD	positive	FHC 1–2 mm lateral to DAE	0.4–0.6	8–10°
moderate – severe CHD	positive	FHC ≥ 3 mm lateral to DAE	> 0.6	> 12°
severe CHD	positive	FHC ≥ 3 mm lateral to DAE	> 0.8	> 15°

* FHC/DAE – position of the femoral head centre to the dorsal acetabular rim
**DARA – dorsal acetabular rim angle

Tab. 2. Evaluation of the prognosis and diagnosis agreement

[1]made according Vezzoni in 4 months old dogs; [2]made on the basis of FCI method in 12 months old dogs; [3]positive result means that our prognosis for development and final score for canine hip dysplasia (CHD) were in agreement, negative result means disagreement between prognosis and diagnosis

List of patients	Prognosis of the hip development[1]	Final hip dysplasia degree[2]	Result[3]
1	Near normal hip joints	B	Positive
2	No signs of CHD	C	Negative
3	Near normal or mild CHD	C	Positive
4	No signs of CHD or near normal hips	B	Positive
5	Moderate or severe CHD	E	Positive
6	No signs of CHD	A	Positive
7	No signs or near mormal	B	Positive
8	Near normal or mild CHD	C	Positive
9	Mild CHD	A	Negative
10	Near normal or mild CHD	C	Positive

the longitudinal axis of the spine and another one through the point of contact of the dorsal acetabular rim with femoral head and the resulting angle for the respective joint is read by means of a goniometer (Fig. 6).

The tangential angle was determined as an angle between two lines passing through the point of contact of the femoral head and dorsal acetabular rim (Fig. 7).

Statistical analysis of the results

We calculated Pearson correlation coefficients for individual paired measurements of the Norberg angle, distraction index and FCI score obtained for dogs of the same age (NA4/FCI4, NA4/DI4, FCI4/DI4; NA12/FCI12, NA12/DI12, FCI12/DI12) and for individual paired measurements at different ages of dogs (NA4/FCI12, NA4/DI12, NA4/NA12, FCI4/NA12, FCI4/DI12, FCI4/FCI12, DI4/NA12, DI4/FCI12, DI4/DI12). Then we evaluated statistical significance for individual paired measurements.

RESULTS

On the basis of the measurements obtained from radiographs, taken at the age of 4 months (values of the distraction index, dorsal acetabular rim angle and position of the femoral head centre to the dorsal acetabular rim (FHC/DAE)), we determined prognosis of further development of the hip joint according to the Vezzoni (Tab. 1).

The X-ray examination carried at the age of 12 months allowed us, on the basis of FCI method, to make the final diagnosis. Subsequently we compared the prognosis with the final diagnosis (Tab. 2) and all cases in which concurrence was found we considered as positive (an agreement between evaluations based on early diagnosis of canine hip dysplasia and evaluation conducted after reaching the age of 12 months). An agreement was detected in 80 % of cases (8/10). In one case we made false positive prediction (prediction: mild CHD; final degree: A) and in another one false negative prediction (prediction: no signs for development of CHD; final degree: C).

The highest correlation was observed between values DI12/FCI12 (0.86) and DI4/FCI2 (0.78); it means that on the basis of DI values, obtained by measurements at the age of 4 months, it is possible, with 78 % probability, to predict the final FCI score in 12 months old dogs) with extremely high significance ($P < 0.0001$) and the lowest correlation between parameters NA12/DI12 (-0.18) and NA12/DI4 (-0.38) also with extremely high significance ($P < 0.0001$) (Tab. 3).

DISCUSSION

Early diagnosis of CHD presents a new strategy within the programmes striving to reveal and eradicate it. The disadvantage of the up-to-date method based on

Tab. 3. Pearson correlation coeficient for evaluation of canine hip joints
* — result is significantly different ($P < 0.0001$); ** — diference is insignificant ($P = 0.5877$)

		4 months old dogs			12 months old dogs		
		FCI	NA	DI	FCI	NA	DI
4 months old dogs	FCI	1	-0.67	0.42	0.45	-0.50	0.44
	NA	–	1	-0.52	-0.52	0.71	-0.49
	DI	–	–	1	***0.78****	-0.38*	***0.82*****
12 months old dogs	FCI	–	–	–	1	-0.42	***0.86****
	NA	–	–	–	–	1	-0.18*
	DI	–	–	–	–	–	1

evaluation of conventional ventrodorsal projection is that one cannot exclude certain degree of subjectivity when performing this evaluation. In addition to that some authors point to low reliability of the Norberg angle, possibility of exchange of dogs, presence of arthritic age-related changes, varying qualification of evaluators and different assessment of a radiograph by the same evaluator (agreement in 48—75 % of cases) (13). Besides that opinions were presented that the standard ventrodorsal position results in extending and application of spiral pressure on the articular capsule which affects reposition of the subluxated femoral head into the *acetabulum* and therefore also evaluation itself (6).

Certain degree of subjectivity at evaluations based on early diagnosis is also obvious. The first essential factor is the experience of the person ensuring fixation and taking X-ray which is very important for obtaining radiographs of certain standard level that could be evaluated objectively. Another important step is the evaluation of radiographs that must be carried out by experienced professionals.

In addition to that certain specialists consider the PennHIP not very specific method. In the 0.3–0.7 DI range there is even 47 % chance of false positive results because not all joints exhibiting passive laxity will be affected by dysplasia (4).

The view of dorsal acetabular rim is a usable supplement to the standard ventrodorsal position because it provides information about the bearing, i.e. the most loaded part of the joint. The key factor in positioning is to ensure superimposition of four pelvic structures which, in the cranial direction, include the following: *ala ossis ilii*, *corpus ossis ilii*, *acetabulum* and *tuber ischii*. This method can be considered more objective compared to the palpation examination.

Some authors stated that the observations of increased laxity, insufficient smoothness of cartilage or other signs of moderate dysplasia were not proved by examination of standard radiographs but in 35 % of such cases the dorsal acetabular rim radiographic view showed symptoms of damage to dorsal acetabular rim (9). We again face the problem of subjective evaluation because significant differences between measurements of the dorsal acetabular slope made by various evaluators were observed (4).

The Ortolani palpation test was used in our experiment only sporadically which prevents us from confirming or rejecting the information about its reliability. However, a study was published according to which the Ortolani method provided most reliable results at the age of 16—18 weeks and the highest reliability of DI and NA based evaluations was observed at the age of 6—10 and 16—18 weeks (1).

From the point of view of application of methods intended for early diagnosis of canine hip dysplasia the most reliable appeared to be the method based on determination of the distraction index. Statistical evaluation of our experiment that included determination of correlation coefficients of the two methods showed high correlation between DI4/FCI12 ($r = 0.78$) and DI12/FCI12 ($r = 0.82$). For comparison, in the study by Smith *et al.* (11) the correlation coefficient for DI4/DI12 was 0.82 (in our experiment $r = 0.86$, i.e. the difference was +4 %), for DI4/OFA12 $r = 0.52$ (difference +26 %) and for DI12/OFA12 $r = 0.67$ (difference +15 %).

However, when the DI value was determined in dogs younger than 16 weeks, the results became unreliable, e.g. in the study on 2 months old German Shepherds (12)).

Early X-ray diagnosis of canine hip dysplasia performed in our study confirmed the conclusions of other authors that it is beneficial to both dog owners and veterinarians and because of that it is justified as one of the actions in the whole complex of measures oriented on detection and eradication of CHD but also in the process of decision taking when the early surgical treatment, intended to affect such development in the canine hip joint, should be considered.

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CYTOGENETIC EFFECT OF PESTICIDES ON CULTIVATED PERIPHERAL LYMPHOCYTES OF CATTLE

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ABSTRACT

We present the results of cytogenetic experiments using two pesticides (insecticide and fungicide) which were added to bovine peripheral lymphocyte cultures at different concentrations. No positive clastogenic effect was observed after 24 h *in vitro* exposure of cattle lymphocytes either to insecticide bendiocarb or to fungicide tolylfluanid. Decrease in mitotic index was shown in both donors at the highest bendiocarb concentration added to the cell cultures, indicating cytotoxic effect of the insecticide. The results with tolylfluanid suggested that the fungicide has an ability to disrupt the cell cycle. Inhibition of functional spindle apparatus formation or action on cellular membranes could be the reasons of statistically significant increase in the frequency of polyploid and aneuploid cells after exposure to this fungicide.

Key words: bendiocarb; cattle; cytogenetic effect; pesticides; tolylfluanid

INTRODUCTION

The pesticides currently used in agriculture include a wide variety of compounds belonging to different chemical classes. Pesticides have been considered potential chemical mutagens and pesticide exposure recognized as an important environmental risk factor associated with an increase in genotoxicity indices and cancer development in humans as well as in experimental animals (11). Livestock such as cattle and sheep can be exposed to pesticides via pasture. Cytogenetic data obtained by examination of animal peripheral lymphocytes are useful for the assessment of early effects of chemical agents and genetic risk.

In our study, cytogenetic effects of bendiocarb (N-methylcarbamate insecticide) and tolylfluanid (phenylsulphamide fungicide) were evaluated in cultivated bovine lymphocytes after 24 h exposure.

MATERIAL AND METHODS

Whole blood specimens from two clinically healthy bull donors (Slovak spotted cattle, 6—8 months old) were cultivated for 72 h at 38 °C in 5 ml of RPMI 1640 medium supplemented with L-glutamine and 15 mmol.l^{-1} HEPES (Sigma, St. Louis, MO, USA), 15 % foetal calf serum (Sigma, Chemical Co. St. Louis, MO, USA), antibiotics (penicillin 250 U.ml^{-1} and streptomycin 250 µg.ml^{-1}) and phytohaemagglutinin (PHA, 180 µg.ml^{-1}, Welcome, Dartford, England). Bendiocarbamate (CAS 22781-23-3, chemical name 2,3-isopropylidene-dioxyphenyl methyl carbamate) was added to the lymphocyte cultures at concentrations of 20, 40, 80 and 160 µg.ml^{-1} for the last 24 hours. Tolylfluanid (1,1-dichloro-N-[(dimethylamino) sulfonyl]-1-fluoro-N-(4-methylphenyl)methanesulfenamide), trade name Euparen M/Euparen Multi, Bayer AG, Germany (CAS no. 731-27-1), was dissolved in dimethyl sulphoxide (DMSO, Sigma, St. Louis, MO, USA) (CAS no. 67-68-5) and applied to culture flasks at concentrations of 1.7, 3.5, 8.75 and 17.5 µg.ml^{-1}. The doses were chosen referring to the highest dose causing a reduction in the mitotic index (MI) of more than 50 %. Ethylmethane sulphonate (EMS, Sigma, St. Louis, MO, USA, 250 µg.ml^{-1}) was used as a positive control agent. Chromosome preparations were obtained using the conventional cytogenetic method; 2 h before the harvest, colchicine (Merck, Darmstadt, Germany) was added at a concentration of 5 µg.ml^{-1}. Slides were stained with Giemsa or hybridised with fluorescently labelled probes.

For chromosome aberration (CA) analysis, one hundred well-spread metaphases per donor and concentration were analysed including chromatid breaks (CB), isochromatid breaks (IB), chromatid exchanges (CE) and isochromatid exchanges (IE). Gaps (G) were examined separately. The mitotic index (MI) was calculated as a ratio between the number of cells in mitosis and the total number of 2000 cells. For fluorescent *in situ* hybridisation analysis (FISH), green and orange-labelled whole chromosome painting (WCP) probes, specific for the bovine chromosomes 1 and 5 (BTA1, BTA5) (prepared in Veterinary Research Institute, Brno, The Czech Republic) were used. Overnight hybridisation and washing were performed according to a standard procedure (16). The slides were counterstained in DAPI/Antifade (4´, 6´-diamino-2-fenolindol, Q-BIOgene, Middlesex, UK). A fluorescent microscope Nikon Labophot 2A/2, equipped with dual band pass filter FITC/TRITC, was used for probe visualisation. 250 metaphases were scored per donor and concentration. Chromosome aberrations were described according to PAINT nomenclature (17) and recorded by means of a Nikon digital camera (Coolpix 4500, Nikon). The statistical analysis of FISH results was performed using χ^2 test.

RESULTS

The results of chromosome aberration analysis in bovine peripheral lymphocytes after 24 h exposure to bendiocarb are shown in Table 1. A small elevation of induced chromosome damage (chromatid breaks, CBs) was shown in cells in relation to increasing concentrations ranging from 20 to 80 μg.ml^{-1} in each donor. However, no significant dose-dependence proving the clastogenic effect of bendiocarb was observed. The highest bendiocarb concentration tested (160 μg.ml^{-1}) caused a significant inhibition of mitotic activity in both donors ($P < 0.05$, $P < 0.01$, respectively), reflected in lower values of mitotic indices in comparison with control cultures. Thus only insufficient number of metaphases could be analysed to detect chromosome aberrations after the treatment with the highest insecticide concentration. On the basis of the results of chromosome aberration assay, two concentrations of insecticide (40 and 80 μg.ml^{-1}) were chosen and added to the cell cultures to examine the stable chromosomal aberrations by FISH-WCP. Translocations between BTA1 and BTA5 as well as numerical aberrations, such as polyploidies (mostly tetraploidies, 4n), were also detected after higher bendiocarb exposure. However, the increased level of both translocations and numerical aberrations in exposed bovine lymphocyte cultures did not reach statistically significant values.

The frequencies of chromosomal aberrations induced by the fungicide tolylfluanid in bovine peripheral lymphocytes *in vitro* culture are shown in Table 2. The chromosome aberration assay with conventional Giemsa staining for 24 h showed no positive clastogenic effect. Tolylfluanid induced an increase in the number of chromosomal aberrations at all concentrations tested in each donor, but without statistical significance. The common types of chromosome aberrations were chromosome breaks. The maximum frequency of breaks was found at a dose of 8.75 μg.ml^{-1}. In contrast to the detection of structural aberrations the increases in the number of numerical aberrations were significant. The highest concentrations (8.75 and 17.5 μg.ml^{-1}) also resulted in a significant inhibition of mitotic activity ($P < 0.05$ and $P < 0.01$, χ^2 test).

In case of 24 h tolylfluanid exposure, FISH did not detect statistically significant differences in the frequency of structural aberrations. Compared to controls, the elevated frequency of polyploid (4n) cells was observed at all concentrations tested. A significant increase in frequency of polyploidies and a dose-dependence was shown after exposure to tolylfluanid at concentrations of 3.5, 8.75 and 17.5 μg.ml^{-1} ($P < 0.05$, $P < 0.01$, and $P < 0.001$, χ^2 test).

Table 1. Induction of chromosome aberrations in bovine peripheral lymphocytes exposed to bendiocarb for 24 h

Dose	G	Types of chromosomal aberrations CB	IB	CE	IE	% breaks (± SD)	% MI
Donor 1		**Treatment for 24 h**					
Control	2	1	-	-	-	1.0 ± 0.10	2.8
Bendiocarb (μg.ml^{-1})							
20	4	1	-	-	-	1.0 ± 0.10^{a}	2.9^{a}
40	4	2	-	-	-	2.0 ± 0.14^{a}	2.9^{a}
80	7	5	-	-	-	5.0 ± 0.22^{a}	2.8^{a}
160	3	3	-	-	-	4.0 ± 0.20$^{a, b}$	1.3*
250 μg.ml^{-1} EMS	14	12	-	5	2	26.0 ± 0.64***	1.0**
Donor 2	**Treatment for 24 h**						
Control	5	2	-	-	-	2.0 ± 0.14	3.0
Bendiocarb (μg.ml^{-1})							
20	3	3	-	-	-	3.0 ± 0.17^{a}	2.7^{a}
40	5	3	-	-	-	3.0 ± 0.17^{a}	2.6a
80	9	6	-	-	-	6.0 ± 0.24^{a}	2.0^{a}
160	4	4	-	-	-	6.7 ± 0.25$^{a, c}$	1.2**
250 μg.ml^{-1} EMS	11	14	2	9	1	36.0 ± 0.97***	0.8***

A total of 100 well-spread metaphases of each concentration was determined, when possible.
*, **, *** – significant differences $P < 0.05$, $P < 0.01$, $P < 0.001$, resp. χ2 test; ^{a} – differences were insignificant; $^{b, c}$ – insufficient number of cells (b75, c60 analysed metaphases); CB, IB – chromatid, isochromatid break, CE, IE – chromatid, isochromatid exchange, G – gaps, not included in statistic data

Table 2. Induction of chromosome aberrations in cow peripheral lymphocytes exposed to tolylfluanid for 24 h

Dose	Types of chromosomal aberrations					Percent (%) breaks (± SD)	Percent MI (%)
	G	CB	IB	CE	IE		
Donor 1							
Control (DMSO)	3	2	-	-	-	2.0 ± 0.14	4.2
Tolylfluanid (μg.ml^{-1})							
1.7	3	3	-	-	-	3.0 ± 0.17[a]	3.2[a]
3.5	4	4	1	-	-	5.0 ± 0.22[a]	2.8[a]
8.75	6	6	-	-	-	6.0 ± 0.24[a]	2.2*
17.5	8	4	2	-	-	6.0 ± 0.24 [a]	2.0**
250 μg.ml^{-1}, EMS	12	10	3	2	3	23.0 ± 0.52***	1.0***
Donor 2							
Control (DMSO)	2	2	-	-	-	2.0 ± 0.14	3.9
Tolylfluanid (μg.ml^{-1})							
1.7	4	3	-	-	-	3.0 ± 0.17[a]	2.8[a]
3.5	5	4	-	-	-	4.0 ± 0.20[a]	2.6[a]
8.75	7	6	-	1	-	8.0 ± 0.30[a]	2.1*
17.5	4	5	-	1	-	7.0 ± 0.29[a]	1.6**
250 μg.ml, EMS	10	9	1	1	4	22.0 ± 0.54***	0.8***

A total of 100 metaphases were scored for each treatment.

*** – statistical significance ($P < 0.001$, χ^2 test), [a] – insignificant differences; CB, IB – chromatid, isochromatid breaks, CE, IE – chromatid, isochromatid exchanges, G – gaps, not included in statistical data

DISCUSSION

As far as the carbamate insecticides are concerned it was shown that while some carbamates had no cytogenetic effect either on mitosis or meiosis (18), other group of carbamates included the genotoxic agent and a potential germ cell mutagen (5). In our study, a cytogenetic and possible genotoxic potential of a broad-spectrum of N-methylcarbamate insecticide bendiocarb was examined. Only few data have been published concerning induction of chromosomal aberrations in animal cells exposed to insecticides. Piešová and Valočíková (14) recorded that *in vivo* exposure to bendiocarbamate failed to increase significantly the frequencies of micronuclei in rabbit bone marrow erythrocytes. Interestingly, decrease in bone marrow proliferation was evident in reduction of polychromatic erythrocytes. In our experiment, no significant concentration dependence was observed in relation to induction of chromosomal aberrations. Only the chromatid type of aberrations was detected probably indicating the indirect mode of action of the insecticide (9). The highest bendiocarb dose used (160 μg.ml^{-1}) induced a significant decrease in the mitotic ability of both donor cultures when compared with the controls. This suggests a cytotoxic effect of the agent at this concentration treatment reflected in reduction of MI (> 50 %). Our results resemble those presented by EPA (2); in the absence of S9 activation there was no indication of clastogenicity at 143 μg.ml^{-1}, which was a dose level considered slightly below the excessively cytotoxic dose of 170 μg.ml^{-1}.

It is well known that stable chromosomal changes have been associated with different types of human cancer. Under the conditions of our experiment, two types of translocations were visualised by means of fluorescent labelled probes: translocations between chromosome 5 and other non-labelled chromosomes as well as a translocation of chromosome 1 and chromosome 5. These findings suggest that the insecticide treatment probably had not a potential to induce a sufficient number of breaks following the higher incidence of translocations in treated cells. Besides structural changes, numerical aberrations, such as tetraploid and heteroploid lymphocytes with colour painted chromosomes 1 and 5, were distinguished by FISH WCP; at present it is assumed that one proposed route to aneuploid cancer is through unstable tetraploid intermediate (4). Lin *et al.* (8) reported that N-methylcarbamate insecticides inhibited significantly gap-junctional intercellular communication revealing their potential as non-genotoxic carcinogens. According to these authors, the importance of further studies on the insecticides mentioned, especially on their genetic toxicology, is evident.

Our results, involving the chromosome aberration assay for 24 h exposure to tolylfluanid, indicated only slight elevations in the induction of aberrations with all concentrations tested when compared with the controls. This allowed us to assume rather indirect (epigenetic) mechanisms in the chromosome induction than direct DNA injuries.

Numerical aberrations, polyploidy and aneuploidy, were the next type of aberrations detected by conventional and FISH stained metaphases. Our results indicated that the use of FISH with painting probes was capable of detecting polyploid as well as aneuploid cells occurring at relatively low frequencies. This technique facilitates more rapid and strict analysis than the conventional chromosome painting. As reported by Kirsch-Volders *et al.* (7) and Hagmar *et al.* (6), changes in chromosome number are associated with different stages of carcinogenesis. While aneuploidy plays a specific role in the early stages of carcinogenesis (3), polyploidy is likely to be important in a later stage, in the generation of karyotypic instability (10). Induction

of aneuploidy or polyploidy could trigger apoptosis. The importance of polyploidy as a genotoxic endpoint is still not definite, mainly due to their natural occurrence in some somatic tissues (12). Unlike aneuploidy, which is caused either by chromosome loss during cell division or non-disjunction of chromosomes (13), polyploidy is never caused by DNA damage. The significant increase in the frequency of polyploid and aneuploid cells after the exposure to tolylfluanid could be caused by inhibition of functional spindle apparatus formation or by action on cell membrane (19).

Evaluation of the mitotic index (MI) is an additional check on the potential of the chemical agent. The reduction in MI suggests a cytotoxic/cytostatic effect of chemicals or delay in cell cycle. Our results with tolylfluanid indicated decreased division of cells at concentrations of 8.75 and 17.5 μg.ml^{-1}. This is comparable with a pronounced reduction in the number of cells recorded by EPA (1) after the exposure to tolylfluanid at a concentration of 10 μg.ml^{-1}. Similarly, a cytotoxic effect and suppressed mitogenic activation was observed in sheep lymphocytes after exposure to tolylfluanide-like pesticide dichlofluanid (15).

The results of our cytogenetic experiments with two pesticides did not confirm a positive clastogenic/genotoxic effect after 24 h *in vitro* exposure of bovine lymphocytes either to insecticide bendiocarb or to fungicide tolylfluanid. A decrease in mitotic indices was shown in lymphocytes of both donors at the highest bendiocarb and tolylfluanid doses tested, suggesting cytotoxic effect of the respective pesticides at these concentrations. The evaluation of tolylfluanid effect on cultured bovine peripheral lymphocytes indicated that the fungicide was capable of inducing mitotic exit errors which was reflected in a significant, dose-dependent increase in polyploid cells level.

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SEROLOGICAL DETECTION OF ANTIBODIES TO *Toxoplasma gondii* IN ANIMALS KEPT IN HOUSEHOLDS

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ABSTRACT

The study presents the results of serological examination of animals kept in households. The presence of antibodies against *Toxoplasma gondii* in cats, dogs and rabbits was observed by complement fixation test. Blood specimens were collected from 92 asymptomatic animals. Tests were carried out using a complement fixation test with all titres over 1 : 8 evaluated as positive. Of the 92 examined serum specimens with titres between 1 : 8 and 1 : 64, 47 samples were positive (51.1 %). Of the 32 examined sera 19 (59.4 %) reacted positively and 13 (40.6 %) were negative. Of the 39 cat serum samples 13 were positive (33.3 %) and 26 reacted negatively (66.7 %). Of the 21 rabbit samples, 15 (71.4 %) reacted positively and 6 (28.6 %) were negative. The results obtained indicate that toxoplasmosis is wide-spread among animals kept in households.

Key words: cats; complement fixation test; dogs; rabbits; serological prevalence; *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis is one of the most common worldwide parasitic zoonoses afflicting a broad range of mammals and birds. The aetiological agent is *Toxoplasma gondii* (*T. gondii*) the definite hosts of which are representatives of the family Felidae infected by oocysts from the environment or by tachyzoites and bradyzoites from intermediary hosts, such as all kinds of vertebrates including humans. It is a pantropic cosmopolite and facultatively heterogenic coccidium. Canine and feline toxoplasmosis is a multi-systemic disease; however a latent form of the disease usually develops (2). Dogs may act as a mechanical factor in transmitting toxoplasmosis to humans by rolling in foul-smelling substances and by ingesting fecal material. The fact that 50 % of stray dogs and cats carry *T. gondii* antibodies means that they have been infected and may transmit the parasite. Cats are very important hosts in the epidemiological cycle of *Toxoplasma gondii*, a zoonotic protozoan parasite that can infect humans and many other animal species worldwide. People and especially immunocompromised individuals and pregnant women should observe the hygienic principles not only after contact with soil, cats, before eating, but also after contact with dogs.

The objective of the present study was to update the available information on the serological prevalence of *T. gondii* infection in animals kept in households.

MATERIAL AND METHODS

Animal sera: 92 serum specimens of asymptomatic animals were examined, out of which 32 were dog sera, 39 cat sera and 21 rabbit sera. In the cats and dogs, blood serum specimens were taken from *vena cephalica* and in the rabbits from *vena auricularis*. Before the tests, the blood sera were stored at a temperature of –20 °C.

Serological test: In order to prove the presence of overall antibodies to *T. gondii*, a complement fixation test (CFT) according to Zástěra (12) was employed.

Toxoplasma complement, *Toxoplasma* positive and negative (Imuna a. s., Slovakia), and Hemolytic system (ready to use;

Virion, Switzerland) were used to diagnose *Toxoplasma* antigen KFR. All titres over 1:8 were evaluated as positive when using a twofold dilution.

RESULTS

In 2008 a total of 92 blood serum specimens were examined for the presence of antibodies to *T. gondii* by complement fixation test. A titre of 1 : 8 and all higher titres were evaluated as positive. Of the 92 specimens, the presence of antibodies to *T. gondii* was detected in 47 cases (51.1 %). In CFT, of the 32 examined dog sera titrated at ratios ranging between 1 : 8 and 1 : 64, 19 specimens reacted positively (59.4 %) and 13 (40.6 %) were negative. Of 39 cat serum specimens, titrated at ratios between 1 : 8 and 1 : 64, 13 (33.3 %) serum specimens were positive for antibodies against *T. gondii* and 26 (66.7 %) specimens were negative. Examination of rabbit serum samples showed that 15 out of 21 sera were positive (71.4 %) reaching titres 1 : 8—1 : 64, and 6 (28.6 %) samples were negative (Tab. 1).

Table 1. Presence of anti-*Toxoplasma* antibodies in examined animals

Species	Number of examined specimens	Positive 1 : 8–1 : 64
Dogs	32	19 (59.4 %)
Cats	39	13 (33. 3 %)
Rabbits	21	15 (71. 4 %)
Total	**92**	**47 (51. 1%)**

DISCUSSION

Serological diagnosis of *T. gondii* infections in dogs and cats was investigated by many authors (1, 3, 4, 7, 9, 11). The tests used include Sabin-Feldman test, complement fixation test, indirect haemagglutination, direct agglutination, indirect fluorescent antibody assay and enzyme immunoassay. Demonstration of antibodies by these serological tests just indicates previous infection by *T. gondii.* Laboratory diagnosis of toxoplasmosis requires demonstration of high titres of specific antibodies and increasing antibody levels in two serum samples taken 2 to 4 weeks apart (2). Prevalence of antibodies to *T. gondii* was determined in sera from dogs in Grenada, West Indies. Using a modified agglutination test, antibodies to *T. gondii* were found in 52 (48.5 %) of the 107 dogs, with titers of 1 : 25 in 17, 1 : 50 in 19, 1 : 100 in 7, 1 : 1,600 in 5, and 1 : 3,200 or higher in 4 (3).

Lopes *et al.* (7) conducted a serological survey of antibodies to *T. gondii* in domestic cats from northeastern Portugal by means of modified agglutination test. Three cats had titres of 20 (3.9 %), 18 had titres of 40 (23.7 %) and 55 animals had titres of ≥ 800 (72.4 %). Infection levels were significantly different between cats that lived totally indoors (7.7 %) and those that had access to outdoors (45.4 %) as well as between cats living alone (13.8 %) and those that had contact with other cats (39.4 %). Seroprevalence values in cats fed only commercial canned or dried food (22.9 %) and animals the diet of which included raw or undercooked viscera and/or meat (53.5 %) were also significantly different. Age, habitat and diet were identified as risk factors for the feline *T. gondii* infection by logistic regression analysis. Some control measures were suggested based on these findings (7).

Samples of serum taken during 1986 and 1987 from 244 pet cats and 303 dogs were screened by enzyme-linked immunosorbent assay (ELISA) for antibodies to *Toxoplasma gondii.* It was revealed that 42 % of cats and 23 % of dogs were seropositive (11).

Five hundred and sixty seven sera of healthy house cats were examined for the presence of anti-toxoplasma antibodies by indirect immunofluorescence assay. Twenty-five percent of cats tested positive for IgG and/or IgM. The seroprevalence increased with age from 2 % below 12 months of age up to 44 % in cats 7 years old. The results suggested that *T. gondii* infections are common in house cats and that there is a high chance for a negative cat to seroconvert in its second year of life (1).

Sera of 413 dogs and 286 cats from the Czech Republic were tested for antibodies to *T. gondii* by the indirect fluorescent antibody test. The IgM antibodies to *T. gondii* were found in 10 (2.4 %) dogs and 8 (2.8 %) cats; IgG antibodies were found in 107 (25.9 %) dogs and 126 (44.1 %) cats. Of the dogs, the most exposed group were pet dogs, followed by police dogs; no antibodies were found in laboratory dogs. No significant differences in prevalence were observed between clinically healthy (n = 115) and diseased pet dogs (n = 80) which reached 0.87 % and 1.25 % for IgM, and 33.9 % and 33.75 % for IgG, respectively. Although *T. gondii* is a common parasite in domestic cats and dogs the clinical importance of the zoonosis is low (9).

Figueroa-Castillo *et al.* (4) determined antibodies to *T. gondii* by indirect ELISA in serum samples from domestic rabbits from 3 rabbit farms in Mexico. Antibodies to *T. gondii* were found in 77 (26.9 %) of 286 animals. On the farm with higher rearing standards the seroprevalence was 18.7 % whereas on the farm with medium standard and another family-managed farm the seroprevalence was 39.7 and 33.3 %, respectively. This report was the first report concerning prevalence of antibodies to *T. gondii* in rabbits in Mexico. Although the prevalence found in the present study was within the range reported in other countries, two of the farms revealed a relatively high prevalence which was probably associated with the presence of cats inside rabbit houses (4).

Our study detected higher seroprevalence in dogs and rabbits and lower in cats. In term of infection spreading important factors are animal breeding, contact with another animal and composition and processing of feed. In animals kept in households the most important factors are composition and processing of feed. Feeding raw or undercooked meat or offal to household animals plays the main role in spreading of infection. Important components of rabbit feed are fruit and vegetables. Fruit and vegetables contaminated with infected soil and inadequately washed are an important source of *Toxoplasma* oocysts.

The role of domestic rabbits in epidemiology of toxoplasmosis in humans has not been established in detail, but is probably important. Although some authors treat this role marginally, others include rabbits among animal species acting as a major source of infection for humans (10). Ishikawa *et al.* (5) described the case of cervical toxoplasmosis transmitted from rabbit to man. Nevertheless, there is a lack of controlled epidemiological studies on the degree of correlation between the prevalence of toxoplasmosis in rabbits and humans having contacts with these animals (5).

Among laboratory diagnostic techniques, a complement fixation test is one of the most frequently employed techniques for detecting antibodies to *T. gondii*. Results acquired by complement fixation tests in examination for the presence of antibodies against *T. gondii* antigens in the serum specimens of infected animals facilitate the interpretation of such results. The level of overall antibodies in a CFT significantly correlates with the dynamics of IgM and IgA antibodies. A titre of 1 : 256—512 is significant for the acute phase of infection, whereas titres below 1 : 128 point to the chronic or latent course of the disease. With respect to determination of Toxoplasma infection by serological examination, CFT is of greater informative value in comparison with the same requirement related to IgG antibodies (8).

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RESEARCH AND DEVELOPMENT OF A SYNTHETIC QUALITY INDICATOR FOR RAW MILK ASSESSMENT

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ABSTRACT

Raw milk quality (RMQ) is an important factor in milk food chain safety. A marked improvement was observed in both compositional milk quality indicators (MQIs) and especially health and hygienic MQIs during the reference period. A good possibility for improvement is in somatic cell count (SCC), but a consistent link of RMQ to the farmer price (FP) is essential. It was underestimated in the Czech Republic. The aim was to create a new synthetic RMQ indicator (SQSM) from various MQIs for consistently including each RMQ change into FP. An analysis of the properties of MQIs was performed: comparison of mean values of MQIs; exploratory analysis as normality testing of RMQ data files. MQIs were divided into 2 groups without and with the necessity of original data transformation before synthesis into the SQSM. A proposal of evaluation algorithm for synthesis of MQIs in relative MQI (SQSM) was made and validated for use at FP modification. The SQSM file showed an acceptable normality of data frequency distribution, obliqueness −0.23 and acuteness 3.60 ($P < 0.05$) the deviation of which is practically negligible. Milk FP premiums and penalties could be balanced by SQSM.

Key words: bulk milk; data frequency distribution normality; exploratory analysis; milk quality; raw milk; somatic cell count; total mesophilic bacterial count

INTRODUCTION

The raw milk quality (RMQ) is a crucial factor in dairy food chain safety (5, 6, 7, 8, 16, 36, 37, 38). The regular RMQ control system satisfies the important social order (2, 3). Therefore the current RMQ investigation and its payment are very important measures. More authors (4, 12, 23, 24, 25, 26, 35, 39) were concerned with questions of the payment of raw cow milk in accordance with its quality or production economy. It means payment with direct consistent link of the purchase price to the values of the RMQ indicators (MQIs) from different points of view. For instance, in terms of the rules and links of the farmer price construction according to supplier-processor contracts, which are the main instruments for the raw milk quality growth. Some of the rules are in other publications (10, 28, 34). Others (13, 18) were concerned with the possibility of creating a new, consistent, synthetic, relative quality indicator of raw cow milk for every change of its quality to be taken into account in the purchase price. For a further improvement of milk quality indicators a consistent link of the raw milk quality to the farmer price is essential. This fact was often underestimated in the Czech Republic (CR; 13, 18). In the framework of the official payment system, an inconsistent and very inexpressive price differentiation according to raw milk quality has occurred too often in recent years. This stated fact was valid first of all within the standard milk quality. However, the higher raw milk quality usually means extra production costs for farmers (material, equipment, education of the staff). So the situation was often imbalanced from the previously mentioned point of view. This situation should be improved.

This study focused on properties and behaviour of the MQI data files to find and develope the relevant rules for construction of a synthetic raw milk quality indicator (SQSM), which will include a number of individual RMQ indicators (MQIs). This parameter has to express the RMQ in an objective way.

The aim of an algorithm for SQSM which will be searched for is to create a new relative indicator capable of reflecting all quality changes in the price. It has to be flexible in terms of a number of the possibly included MQIs. For this reason the study was aimed at statistical exploratory analysis of the one-dimensional data files about real MQIs in terms of dynamic evaluation of data frequency distribution (FD) and its incidental normality.

MATERIALS AND METHODS

Reference sets of bulk milk samples

Data on the bulk milk samples were obtained monthly from commercial dairy herds for the regular milk quality determination. This was within the official milk payment system during years 1994 and 2003. The samples were analysed by the accredited milk laboratory according to the relevant standard operation manuals. The milk samples came from both the milked populations of dairy cows in the CR, Holstein cattle and Bohemian Spotted cattle. Different numbers of milk samples were investigated for different indicators. The maximum sample number was for milk freezing point and some other milk parameters (n = 72 607) and the minimum for free fatty acids (n = 11 540). The data sets of 1994 and 2003 were slightly different as to the milk quality indicators, which were measured regularly.

Raw cow milk quality in terms of legislation standard changes and reference period

Discrimination limit values according to the valid standards (EEC 92/46; Regulation 853/2004 and CSN 57 0529) were used. The legislative standard discrimination limit (SDL) for the somatic cell count was changed during the mentioned period. There was a limit of ≤ 400 ths.ml^{-1} for the first quality class by the end of 1994 (there also existed a lower class for the ≤ 500 ths.ml^{-1} standard quality). From the beginning of 1995 the limit ≤ 400 ths.ml^{-1} (ths. = thousands) was already valid for the standard raw milk quality, which means that the quality criterion was stricter. The total mesophilic bacteria count standard discrimination limit (SDL) was made more restrictive in a very marked way. In 1994 the value ≤ 300 ths.CFU.ml^{-1} (CFU = colony form unit) was valid for the first class of quality. There were also lower classes of quality (II ≤ 800 ths.CFU.ml^{-1} and III ≤ 2 000 ths.CFU.ml^{-1}). From the beginning of 1995 the limit ≤ 100 ths.CFU.ml^{-1} was valid for the first class and from 1998 for lover standard milk quality. These are the reasons, why the previously mentioned reference period was chosen for the evaluation.

Investigated milk quality indicators with their abbreviations and units

With tested milk quality indicators that were measured and calculated the following listed abbreviations and units have been used: F = milk fat content (g.100 ml^{-1}; %); L = lactose content (monohydrate; g.100 g^{-1}; %); SNF = solids non fat content (g.100 g^{-1}; %); DM = dry matter (g.100 g^{-1}; %; calculated indicator); CP = crude protein (total N × 6.38; g.100 g^{-1}; %); CAS = casein (casein N × 6.38; g.100 g^{-1}; %; methods for nitrogen matters determination according to 15); WP = whey protein content (g.100 g^{-1}; %; calculated indicator); MFP = milk freezing point (°C or m °C × (–1)); SCC = somatic cell count (ths.ml^{-1}; according to 19); F/CP = ratio between fat and crude protein, a calculated indicator of nitrogen/protein metabolism of the dairy cow herd; U = urea concentration (mmol.l^{-1}); SH = titratable acidity (in ml 0.25 mol.l^{-1} NaOH solution); FFA = concentration of milk fat free fatty acids (mmol.100g^{-1}); TMBC = total mesophilic bacteria count (ths.CFU.ml^{-1}); CBC = coli bacteria count (CFU.ml^{-1}); TRBC = thermoresistent bacteria count (CFU.ml^{-1}); PBC = psychrotrophic bacteria count (CFU.ml^{-1}); RIS = occurrence frequency of residues of inhibitory substances ((+/-) % of +; occurrence of antibiotic drugs); MFA = milk fermentation ability by dairy noble culture (in ml 0.25 mol.l^{-1} NaOH solution). The relevant standards were used for analysis of bulk milk samples in the routine laboratory system of raw milk quality control. The results of raw milk quality development during the reference period were commented upon below.

Calculations and statistical procedures

The large data file was validated by determination of the discrimination limits for all the MQIs calculated (mostly x ± 1.96 or 2.58 × sx, which included 95 or 99 % probability). This data filtration should not let through wrong, improbable values for subsequent evaluation, but the data files include also values out of the legislative framework of standard RMQ (e.g. TMBC > 100 ths.CFU.ml^{-1} or SCC > 400 ths.ml^{-1} and so on). The data files of milk quality indicators were divided into two parts for the evaluation: file I contained validated, nonstandard data, in other words with values outside the SDLs; file II contained the validated, standard data, only with values within the range of relevant SDLs, which were mostly in accordance with CSN 57 0529. Set II, e.g. for milk freezing point it means with the suspicion on a foreign water addition. The statistical evaluation of the data files was performed for individual months because of the month payment system for raw milk. The main statistical characteristics, such as the median (m), arithmetical (x) and geometrical means (xg), standard deviation (sx) and variation coefficient (vx) were calculated. If necessary, the MQI (SCC and microbiological indicators in this case) data were transformed (logarithmically $\log_{10}$ or other kind of transformation such as Box-Cox's) before the evaluation (1, 14, 20, 21, 27, 29, 31, 32, 33, 40). The various statistical mean values, such as x, xg and m, and the differences between them were compared. The exploratory analysis in terms of evaluation of the normal data frequency distribution of files of MQI values was performed. The obliqueness (a_3) and acuteness (a_4) of MQI data files were evaluated as well (14, 27).

The mentioned synthetic RMQ indicator (SQSM) was calculated according to the research and development results as follows: DX = (IND – x)/sx, where: IND is the individual value of MQI of supplier, x is month arithmetical average of MQI of relevant milk suppliers including IND and sx its standard deviation; SQSM is the sum of logically oriented (according to RMQ growth) DXs which come from relevant milk quality indicators of identical raw milk delivery. SQSM as a sum can also be divided by the number of MQIs, which is valid in this case (it means, there are possible two kinds of expressions of

Table 1. The comparison (file I) of mean values of the compositional milk quality indicators (MQIs)

MQI	1994			2003		
month	x	m	dI	x	m	dI
F 1	4.11	4.11	0.00	4.17	4.16	0.24
F 7	3.92	3.92	0.00	3.85	3.84	0.26
CP 1	3.38	3.38	0.00	3.42	3.42	0.00
CP 7	3.25	3.26	-0.31	3.27	3.27	0.00
L 1	4.73	4.74	-0.21	4.93	4.94	-0.20
L 7	4.78	4.79	-0.21	4.98	4.99	-0.20
CAS 1				2.51	2.52	-0.40
CAS 7				2.37	2.37	0.00
SNF 1	8.93	8.93	0.00	8.91	8.92	-0.11
SNF 7	8.73	8.75	-0.23	8.73	8.74	-0.11
U 1	2.73	2.56	6.23	3.22	3.18	1.24
U 7	3.95	3.91	1.01	4.15	4.13	0.48

x – arithmetical mean; m – median; dI – difference x-m in %, where the first term of the difference (the minuend) is equal to 100 %; MQI – milk quality indicator; F – fat, %; CP – crude protein, %; l – lactose, %; CAS – casein, %; SNF – solids non fat, %; U – urea, mmol.l^{-1}; abbreviations are valid also for the following tables and figures

SQSM, sum and/or average). As many statistical evaluations and tests were performed it is not possible to demonstrate all the results here. For a demonstration of the procedures used and results obtained, two months (January (1) and July (7), typical of winter and summer seasons) were selected as interesting examples of situation in 1994 and 2003. Besides that only the relevant MQIs were chosen according to the theoretical and practical requirements to ensure clear result demonstration.

Principal methodological steps of SQSM derivation

The research and development of the SQSM consisted of the following steps:

1. Existence of the hypothesis that it is possible to solve the consistent link between raw milk quality and milk purchase price by the synthetic RMQ indicator (SQSM) and that SQSM can include various MQIs (all their changes) in one magnitude with normal data FD and with the possibility of taking into account the technological and health safety demands. The presupposed penalties of the milk price could be balanced by equal relevant premiums in the case of SQSM normal data frequency distribution (13).

2. Analysis of the state and dynamics of properties of the raw milk quality indicators in the CR. The selected real data files of MQIs were evaluated as a model. The changes in legislative SDLs of the principal milk quality indicators were included in a selected reference period. The raw milk quality state and development were compared and commented on during this period. The various mean values of milk quality indicators were compared to obtain knowledge and confirmation for data transformation necessity at the milk quality indicators data synthesis into the SQSM value.

Table 2. The comparison (file I) of mean valu es of the health and hygiene quality milk indicators (MQIs)

MQI	1994						2003					
month	x	m	xg	dI	dII	dIII	x	m	xg	dI	dII	dIII
SCC 1	241	216	204	10.37	15.35	5.56	228	210	199	7.89	12.72	5.24
SCC 7	298	271	253	9.06	15.10	6.64	271	248	234	8.49	13.65	5.65
TMBC 1	178	64	75	64.04	57.87	-17.19	36	18	18	50.00	50.00	0.00
TMBC 7	292	98	112	66.44	61.64	-14.29	48	21	22	56.25	54.17	-4.76
CBC 1	1391	1000	1131	28.11	18.69	-13.10	112	10	26	91.07	76.79	-160.00
CBC 7	1612	1000	947	37.97	41.25	5.30	211	40	53	81.04	74.88	-32.50
TRBC 1	2991	600	724	79.94	75.79	-20.67	1113	500	521	55.08	53.19	-4.20
TRBC 7	2220	800	779	63.96	64.91	2.63	1002	300	413	70.06	58.78	-37.67
PBC 1	48376	13000	13314	73.13	72.48	-2.42	3472	1000	1715	71.20	50.60	-71.50
PBC 7	42854	14000	13354	67.33	68.84	4.61	3858	2000	2251	48.16	41.65	-12.55

x – arithmetical mean; m – median; xg – geometrical mean; dI – difference x-m in %, dII – difference x-xg in % and dIII – difference m-xg in %, where the first term of the difference (the minuend) is equal to 100 % always; SCC – somatic cell count, ths.ml^{-1}; TMBC – total mesophilic bacteria count, ths.CFU.ml^{-1}; CBC – coli bacteria count, CFU.ml^{-1}; TRBC – thermoresistant bacteria count, CFU.ml^{-1}; PBC – psychrotrophic bacteria count, CFU.ml^{-1}; abbreviations are valid also for the following tables and figures

3.Exploratory analysis in terms of evaluation of the normal frequency distribution of selected data files of MQIs during the chosen reference period. This step was also performed so as to take into account the further adequate work with values of mis.

Table 3. The comparison (file I) of mean values of the log of health and hygiene MQIs

log MQI	1994			2003		
month	x	m	dI	X	m	dI
SCC 1	2.3091	2.3345	-1.10	2.2986	2.3222	-1.03
SCC 7	2.4038	2.4330	-1.21	2.3684	2.3945	-1.10
TMBC 1	1.8731	1.8062	3.57	1.2598	1.2553	0.36
TMBC 7	2.0478	1.9912	2.76	1.3462	1.3116	2.57
CBC 1	3.0536	3.0000	1.76	1.4201	1.0000	29.58
CBC 7	2.9763	3.0000	-0.80	1.7243	1.6021	7.09
TRBC 1	2.8598	2.7782	2.85	2.7166	2.6990	0.65
TRBC 7	2.8917	2.9031	-0.39	2.6162	2.4771	5.32
PBC 1	4.1243	4.1139	0.25	3.2343	3.0000	7.24
PBC 7	4.1256	4.1461	-0.50	3.3524	3.3010	1.53

4.Proposal of the evaluation algorithm construction (SyntQLact 2006) for synthesis of various individual milk quality indicators into SQSM. It has to be insensitive to the number of included milk quality indicators.

5.Validation of the evaluation algorithm practicability for synthesis of the raw milk quality indicator (SQSM) for consistent modification of the farmer price and SQSM exploratory analysis and effects of its data transformations. There should be an independence (no significant correlation) of SQSM on the raw milk delivery volume because of the presupposition of its required error-free functions.

RESULTS AND DISCUSSION

Comments on real milk quality development during the reference period

A positive development of cow raw milk quality due to the legislative changes in the SDLs of milk quality indicators (MQIs) was stated in the CR (file I). A marked improvement was observed in both compositional indicators and especially health and hygiene indicators (methodology step 2), e. g. the arithmetical means of the TMBC were 203.8 $ths.ml^{-1}$ in 1994 and 44.5 $ths.ml^{-1}$ in 2003 ($P < 0.01$). A treee fold reduction corresponded to 78.2 %. A ten fold reduction in the CBC was found ($P < 0.01$). Also the decrease of the occurrence frequency in the RIS from 0.64 to 0.24 % was very important ($P < 0.01$). A stagnation was observed in somatic cell count (x = 272

Table 4. The comparison (file I) of mean values with retransformed BC mean value of the health and hygiene quality milk indicators (MQIs), such as SCC, TMBC and PBC

	SCC 2003						TMBC 2003						PBC 2003					
month	x	m	x_g	$F_{retrans}$	dIV	dV	x	m	x_g	$F_{retrans}$	dIV	dV	x	m	xg	$F_{retrans}$	dIV	dV
1	228	210	199	204	2.86	-2.51	36	18	18	16	11.11	11.11	3472	1000	1715	-	-	-
2	246	227	215	222	2.20	-3.26	36	15	17	13	13.33	23.53	7099	3000	2803	2147	28.43	23.40
3	245	228	216	224	1.75	-3.70	39	16	18	14	12.50	22.22	5057	2000	2539	1672	16.40	34.15
4	255	238	225	233	2.10	-3.56	40	16	18	14	12.50	22.22	10993	2000	3111	1807	9.65	41.92
5	261	242	228	236	2.48	-3.51	53	22	25	21	4.55	16.00	3980	1000	2026	1172	-17.20	42.15
6	288	267	251	260	2.62	-3.59	56	25	28	25	0.00	10.71	8443	2000	2757	2061	-3.05	25.24
7	271	248	234	242	2.42	-3.42	48	21	22	19	9.52	13.64	3858	2000	2251	1651	17.45	26.65
8	303	284	265	277	2.46	-4.53	49	21	23	20	4.76	13.04	28276	2000	3095	1841	7.95	40.52
9	280	260	247	255	1.92	-3.24	43	19	20	17	10.53	15.00	7839	1000	2332	-	-	-
10	253	236	224	230	2.54	-2.68	36	17	18	15	11.76	16.67	10985	1000	2570	1165	-16.50	54.67
11	256	234	225	230	1.71	-2.22	49	22	24	21	4.55	12.50	5365	2000	2839	1828	8.60	35.61
12	254	238	224	231	2.94	-3.13	43	20	22	19	5.00	13.64	12650	3000	4022	2801	6.63	30.36

$F_{retrans}$ – retransformed values of BC transformation; dIV – m-$F_{retrans}$ in %; dV – xg-$F_{retrans}$ in %

Table 5. The comparison (file I) of mean values (x and m) of BC transformation values of health and hygiene MQIs, such as SCC, TMBC and PBC

	SCC 2003			TMBC 2003			PBC 2003		
month	x	m	dI	X	M	dI	x	m	dI
1	9.20	9.27	-0.76	1.66	1.71	-3.01	-	-	-
2	10.17	10.25	-0.79	1.27	1.30	-2.36	1.65	1.65	0.00
3	12.26	12.34	-0.65	1.39	1.43	-2.88	0.91	0.91	0.00
4	12.06	12.15	-0.75	1.35	1.38	-2.22	1.00	1.00	0.00
5	11.04	11.13	-0.82	2.04	2.05	-0.49	0.25	0.25	0.00
6	12.27	12.38	-0.90	2.29	2.29	0.00	1.42	1.42	0.00
7	10.62	10.71	-0.85	1.84	1.88	-2.17	1.00	1.00	0.00
8	15.13	15.28	-0.99	1.96	1.98	-1.02	1.11	1.11	0.00
9	11.09	11.16	-0.63	1.76	1.79	-1.70	-	-	-
10	10.09	10.17	-0.79	1.55	1.59	-2.58	0.23	0.23	0.00
11	9.20	9.25	-0.54	2.07	2.08	-0.48	1.00	1.00	0.00
12	10.42	10.52	-0.96	1.89	1.92	-1.59	1.96	1.96	0.00

Table 6. The normality investigation (no = P ≤ 0.05) of data files (II and I) of some compositional MQIs

II MQIs 2003					I MQIs 2003				
MQI			normality		MQI			normality	
month	a_3	a_4	a_3	a_4	month	a_3	a_4	a_3	a_4
SNF 1	0.30	3.26	no	no	**SNF 1**	-0.33	3.81	no	no
SNF 7	0.64	3.50	no	no	**SNF 7**	-0.20	3.61	no	no
CP 1	0.00	3.67	**yes**	no	**CP 1**	0.00	3.67	**yes**	no
CP 7	0.08	3.56	no	no	**CP 7**	0.06	3.6	**yes**	no
F 1	0.80	5.11	no	no	**F 1**	0.26	5.66	no	no
F 7	0.73	4.25	no	no	**F 7**	0.23	4.33	no	no
					L 1	-0.77	4.70	no	no
					L 7	-0.63	4.72	no	no
					CAS 1	-0.06	3.69	**yes**	no
					CAS 7	0.06	3.59	**yes**	no
MFP 1	0.59	4.32	no	no	**MFP 1**	-0.55	5.97	no	no
MFP 7	1.32	7.83	no	no	**MFP 7**	-0.03	7.46	**yes**	no
					U 1	0.09	2.18	no	no
					U 7	0.02	2.33	**yes**	no

MFP – milk freezing point; a_3 – obliqueness; a4 – acuteness; abbreviations are valid also for the following tables and figures

and xg = 228 ths.ml^{-1} in 1994; x = 252 and xg = 230 ths. ml^{-1} in 2003). The current quality was comparable or better than in developed countries with large dairy cow herds. A reserve for the next improvement of the raw milk quality was seen particularly in the somatic cell count in the CR. Despite this fact one can conclude that milk production and processing chain was probably the safest of those considered for comparison (17). Good results regarding the occurrence of various risky micro-organism groups in materials such as cow feed, excrements and raw milk found under mean conditions of model farms in the CR were reported in a previous article (16). According to the Germany Official results of survey conducted in Germany (AFEMA, 3) showed that milk and milk products pertained to the safest food on the market. The compositional indicators such as F, CP, L, DM and SNF were slightly increased which can indicate better dairy herd nutrition on the one hand and also potentially higher yield of dairy technological procedures (cheese making, etc.) on the other hand. Between 1994 and 2003 the urea also increased at least by a third (from 3.00 ± 1.07 to 3.89 ± 1.36 mmol.l^{-1}; P < 0.01). This can be linked to more intensive nitrogen nutrition and to relative lack of energy in cow rations.

Analysis of the state and dynamics of statistical characteristics of the raw MQIs

1. An important step (methodology step 2) was the comparison of selected mean values for the data files of the MQIs, e.g. x, xg and m. Such a comparison is essential for the confirmation of the main presumptions and hypotheses in terms of the possibility of data synthesis of the MQIs into SQSM. There were small (< 7 %), visible (7–15 %) and essential (> 15 %) differences between mean values of the individual MQIs (file I). The higher differences are probably determined first of all by the deviations of the real FD from the normal FD of data files. The comparison indicated the necessity of the raw MQI data transformations. Because of relativem small differences in dI values (Tab. 1) the transformations were not necessary for such MQIs as F, CP, L, SNF, CAS, and U (x was very often quite comparable or equal to the m). Contrary to this (Tab. 2) the transformations were necessary for other MQIs (such as SCC, TMBC, CBC, TRBC, PBC) because of higher relative difference in values dI and dII and because of lower (sometimes, particular in SCC and TMBC) relative differences in dIII. Higher differences could be found usually between the x and xg in comparison with the m (especially in SCC). In TMBC, CBC, TRBC and PBC the median

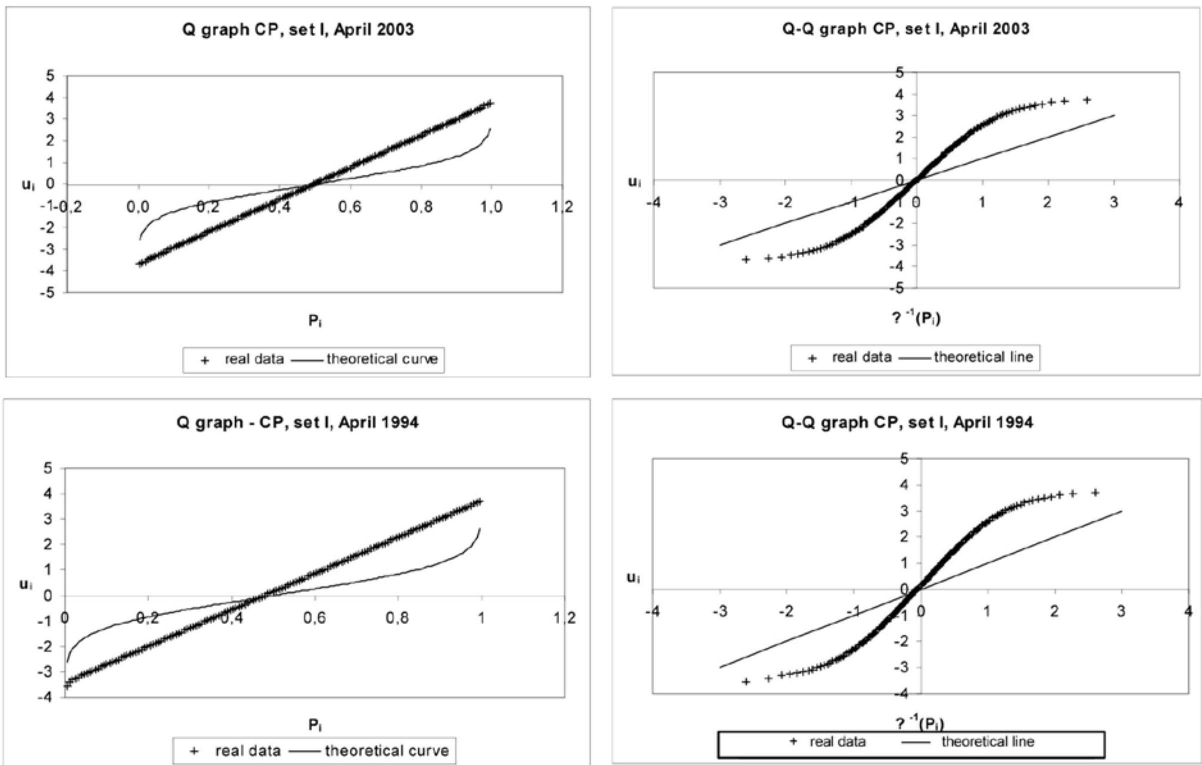

Fig. 1. The exploratory analysis of real frequency distribution (FD) of selected one-dimensional model data files in comparison to the normal standard FD by the quantil and quantil-quantile graph for milk indicator CP
ui – standard quantils of original data; P(i) – order probability;
ui – standard quantils of original data; Φ-1(P) – theoretical quantils of normal standard distribution

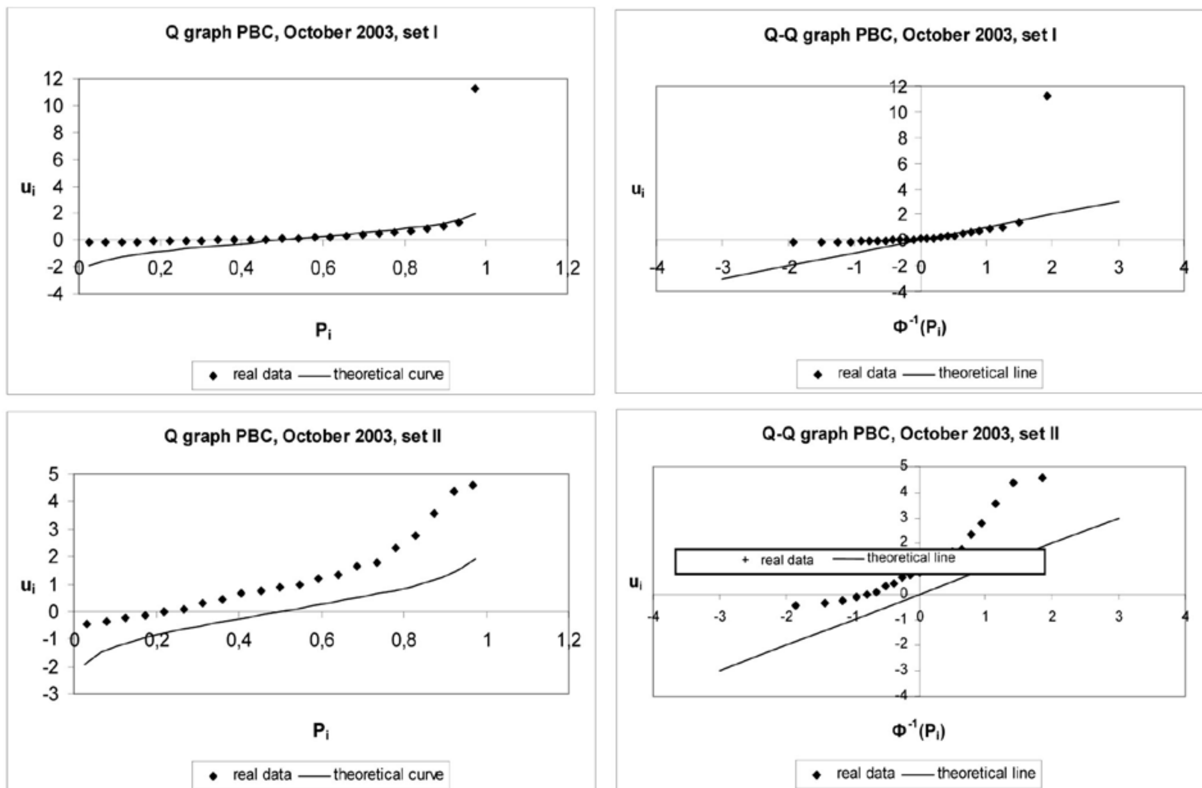

Fig. 2. The exploratory analysis of real FD of selected one-dimensional model data files in comparison to the normal standard FD by the quantil and quantil-quantile graph for milk indicator PBC

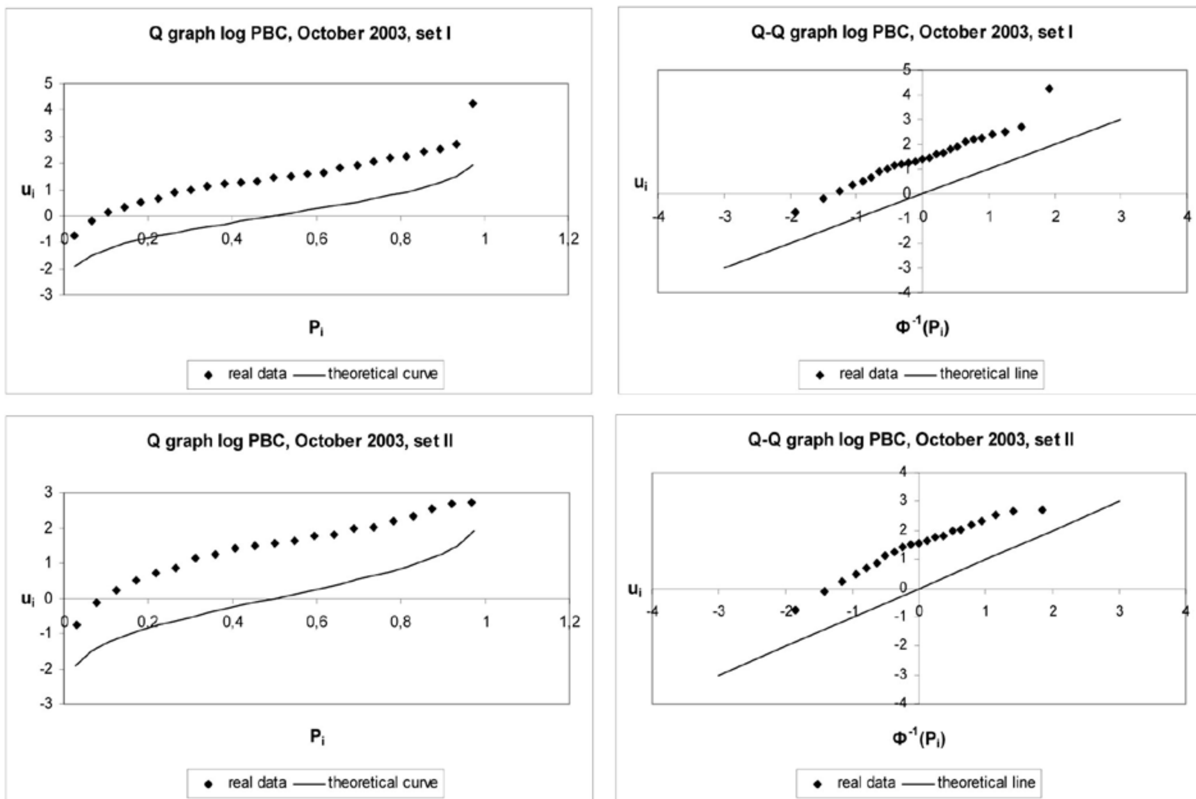

Fig. 3. The exploratory analysis of real FD of selected one-dimensional model data files in comparison to the normal standard FD by the quantil and quantil-quantile graph for milk indicator log PBC

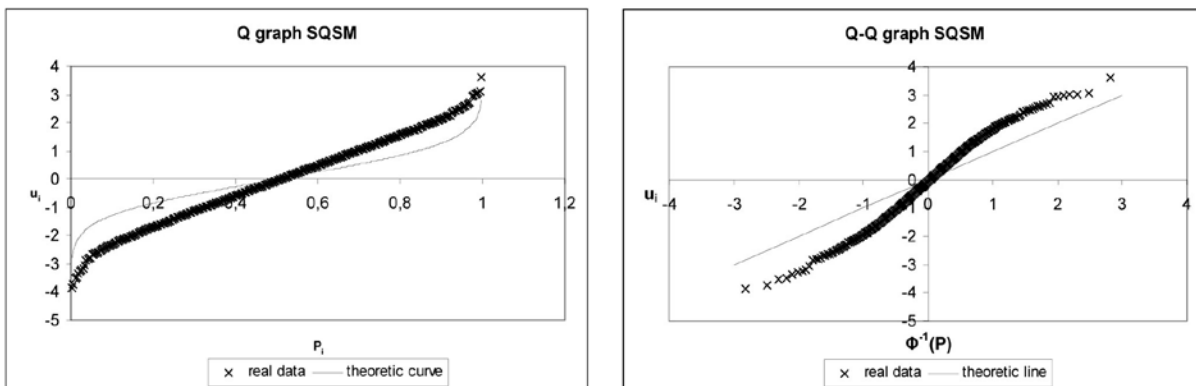

Fig. 4. The exploratory analysis of real FD of one-dimensional model data file in comparison to the normal standard FD by the quantil and quantil-quantile graph for SQSM indicator (January, 2003, file I)

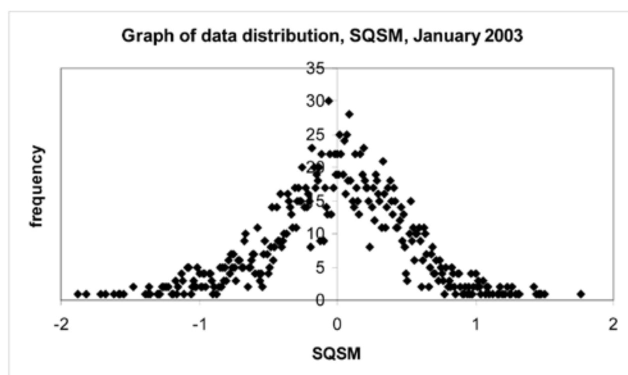

Fig. 5. The real frequency distribution of the derived SQSM indicator file

was mostly lower than xg. As could be seen for the chosen MQIs the TMBC and PBC, there were visibly smaller relative differences (Tab. 3) between the x and m of the logarithmically transformed values than in the original values (Tab. 2). The relative differences in the original values (Tab. 2) of MQIs varied around 70 % and with the transformed values (Tab. 3) mostly under 10 % and in the majority of cases around 3 %. The chosen examples are shown in the whole profiles in Tables 1, 2 and 3. This involves the knowledge for the proposal of the mentioned relevant evaluation of SQSM algorithm. The other milk quality indicators, such as RIS, SH and

Table 7. The normality investigation of data files (II and I) of some health and hygiene MQIs

II MQIs 1994 (a) 2003 (b)					I MQIs 1994 (a) 2003 (b)				
MQI			normality		MQI			normality	
month	a_3	a_4	a_3	a_4	month	a_3	a_4	a_3	a_4
SCC 1, b	0.18	2.34	no	no	SCC 1, b	2.17	13.91	no	no
SCC 7, b	0.01	2.05	yes	no	SCC 7, b	1.90	10.86	no	no
SCC 1, a	0.18	2.18	no	no	SCC 1, a	2.11	11.65	no	no
SCC 7, a	-0.13	2.09	no	no	SCC 7, a	1.65	8.19	no	no
TMBC 1, b	1.97	6.95	no	no	TMBC 1, b	14.55	245.70	no	no
TMBC 7, b	1.63	5.20	no	no	TMBC 7, b	11.91	174.91	no	no
TMBC 1, a	0.46	2.07	no	no	TMBC 1, a	7.53	71.99	no	no
TMBC 7, a	0.06	1.96	yes	no	TMBC 7, a	5.27	33.01	no	no
PBC 1, b	3.86	19.17	no	no	PBC 1, b	5.14	35.17	no	no
PBC 7, b	2.90	13.88	no	no	PBC 7, b	5.59	43.94	no	no
PBC 1, a	1.16	3.37	no	no	PBC 1, a	7.06	77.17	no	no
PBC 7, a	1.06	3.14	no	yes	PBC 7, a	5.48	40.49	no	no
CBC 1, b	3.81	20.89	no	no	CBC 1, b	22.01	670.66	no	no
CBC 7, b	2.45	9.05	no	no	CBC 7, b	11.43	247.19	no	no
TRBC 1, b	1.09	3.38	no	yes	TRBC 1, b	14.67	244.86	no	no
TRBC 7, b	1.69	5.03	no	no	TRBC 7, b	6.88	58.90	no	no

count of sporulating microorganisms (CSMs) were not included into SQSM by the synthesis: SH because of no one way trend for clear deterioration of RMQ; residues inhibitory substances and CSMs because of plus and minus result variant occurrence only. These milk quality indicators decide about the milk standard and nonstandard quality primarily. Nonstandard quality can be penalized for the concrete day of finding. It is evident that Box-Cox's (BC) transformation (27) decreased the month differences dIV (Tab. 4) in comparison to dIII (Tab. 2; 2.64 versus 5.45 % for SCC) and dI-BC (Tab. 5) in comparison to dI-log (Tab. 3; –0.81 versus –1.07 % for SCC). In general, the mentioned was similar in all selected MQIs, it means in SCC, TMBC and PBC (during all months of the year). However, every month was represented by a different value of the control term of the BC transformation (Tab. 9). This was chosen due to optimalization. The mentioned fact shows a good propriety of Box-Cox's transformation for approach to the data frequency distribution normality. However, the problem continues just in the necessity of the individual modification of Box-Cox's control term from case to case (from data file to data file), which is inoperative for the presupposed practical setting.

2. The exploratory analysis was performed in data MQIs (methodology step 3; files I and II). The obliqueness (a_3) and acuteness (a_4) value of the data files of mis, including their quantil-quantile graphs, were interpreted. The compositional MQIs showed usually such behaviour of data FD which was quite near to normality ($a_3 = 0$ and $a_4 = 3$; Tab. 6; Fig. 1). However, these small differences from normality were statistically significant ($P < 0.05$) because of the high number of cases in the MQI data files. Of course, in spite of this significance, the practical importance of such differences was negligible. On the contrary, the health and hygienic MQIs differed mostly from the mentioned character very visibly (Tab. 7; Fig. 2), as expected. The log data transformation approached the

Table 8. The normality investigation of data files (II and I) of log of some health and hygiene MQIs

II log MQIs 2003					I log MQIs 2003				
log MQI			normality		log MQI			normality	
month	a_3	a_4	a_3	a_4	month	a_3	a_4	a_3	a_4
SCC 1	-0.83	3.34	no	no	**SCC 1**	-0.37	3.45	no	no
SCC 7	-0.90	3.35	no	no	**SCC 7**	-0.41	3.31	no	no
TMBC 1	0.30	2.27	no	no	**TMBC 1**	1.03	5.05	no	no
TMBC 7	0.20	2.10	no	no	**TMBC 7**	0.87	3.99	no	no
PBC 1	1.82	5.51	no	no	**PBC 1**	1.88	5.91	no	no
PBC 7	0.80	2.57	no	**yes**	**PBC 7**	0.96	3.26	no	**yes**
CBC 1	1.28	3.50	no	no	**CBC 1**	1.40	4.09	no	no
CBC 7	0.53	2.00	no	no	**CBC 7**	0.60	2.19	no	no
TRBC 1	-0.23	2.16	**yes**	no	**TRBC 1**	0.56	4.16	no	no
TRBC 7	0.38	2.11	no	no	**TRBC 7**	0.73	2.90	no	**yes**

the evaluation algorithm rules for work with the MQI data at their synthesis in SQSM. The above mentioned diversification of milk quality indicators into two groups in terms of necessity of their data transformations or in utility of transformations was specified in this part.

Table 9. The normality investigation of month data files (2003, I) of BC transformation of MQIs

MQI			normality		Box-Cox
month	a_3	a_4	a_3	a_4	control term
SCC 1	0.0015	3.54	Yes	no	0.19
SCC 7	0.00001	3.24	Yes	no	0.22
TMBC 1	-0.0003	2.10	Yes	no	-0.402
TMBC 7	0.0008	2.13	Yes	no	-0.345
PBC 2	-0.0070	1.61	Yes	no	-0.60
PBC 7	0.0690	1.27	Yes	no	-1.00

Table 10. Examples of SQSM values derived from concrete quality of the chosen bulk milk samples

Milk	The quality of raw cow milk from individual suppliers											
supp.	F	CP	SNF	MFP	SCC	log SCC	TMBC	log TMBC	TRBC	log TRBC	RIS	SQSM value
1	4.23	3.56	9.24	523	112	2.0492	31	1.4914	900	2.9542	–	0.173
2	3.32	3.72	9.27	526	341	2.5328	12	1.0792	1200	3.0792	–	-0.160
3	4.28	3.75	9.40	521	287	2.4579	174	2.2405	100	2.0000	–	0.106
4	3.76	3.07	8.56	519	500	2.6990	92	1.9638	64000	4.8062	–	-1.926
x	4.15	3.51	9.05	524	253	2.3494	36	1.2496	1583	2.6114		
sx	0.374	0.183	0.255	5.779	132.9	0.2222	120.6	0.4155	5619	0.5551		

RIS – residues of inhibitory substances; SQSM – synthetic raw milk quality indicator; abbreviations are valid also for other tables and figures

FD towards normality (Tab. 8; Fig. 3) in the files of MQIs usually in a satisfactory way. BC transformation enabled the best approach of files of chosen milk quality indicators (SCC, TMBC and PBC) to normality, especially in terms of more important (for the mentioned purposes) obliqueness of data files ($a_3 = 0$, Tab. 9). BC transformation differs from the uniform logarithmic transformation, dependent on the relevant basis by the need of change and optimalization of its control term according to the character of the concrete data file. In general, the exploratory analysis results confirmed fully the conclusions from the previous paragraph (1) in terms of creation of

SQSM model calculation and validation

All proposed and performed steps at SQSM value derivation strive for obtaining, support and stabilization of its normal FD (methodology steps 4 and 5), e.g. the force of such theoretical rule as Laplace's central limit sentence is presupposed automatically because of the proposed structure of the SQSM creation. As a model example, the concrete SQSM values were derived according to proposed calculation rules for the particular selected bulk milk sample compositions and properties (four deliveries; Tab. 10). No preference (in terms of dairy plant technology purposes) of any MQI was ap-

plied, it means, there were weight values equal to one for all used MQIs. The particular SQSM values varied from –1.926 to 0.173 in this case. The possible theoretical range was from –3.0 to 3.0. In the framework of validation, the results of the SQSM values derivation showed that the log transformation was suitable in the model month file for some real data of MQIs (January, file I, 2003), but the BC transformation was not necessary. Probably no transformation (log or BC) is necessary after the normality test of the SQSM data file ($x = 1.31 \times 10^{-15}$, $sx = 0.486$; $m = 0.021$; min. = –1.88; max. = 1.76; $a_3 = -0.23$, a_3 $sx = 0.053$, $P < 0.05$; $a_4 = 3.60$, a_4 $sx = 0.106$, $P < 0.05$; Fig. 4), as assumed. The derived data file of SQSM values showed quite a good normality of their FD (Fig. 5) in the month file of the real data of MQIs. Although the SQSM data file differed significantly from the normality of FD, first of all because of a high number of cases ($n = 2117$), its real deviation from normality could be seen as negligible, above all for a more important obliqueness (a_3) in this case. The behaviour of the derived SQSM data files could be similar in the practical use. The data transformations are good statistical means but not needed for SQSM file balancing. No preference of any MQI was applied in the model and the combination variant of milk quality indicators was as follows: F, CP, SNF, MFP, log SCC, log TMBC, log CBC and RIS. It was similar as in the case presented in Table 10 with exception of TRBC, which was replaced by CBC in the whole file. The real curve of FD (Gauss curve) is demonstrated in Fig. 5. One could assign the purchase price to these values in practice, according to the results of the relevant commercial negotiations. The milk farmer price premiums could be quite well balanced to equivalence with corresponding penalties by this new RMQ indicator.

CONCLUSION

The presented way of evaluation has not been performed up to now. No study on FD of MQI files for the purpose of obtaining construction rules of a synthetic MQI is known. The results demonstrated the usability of the derived synthetic MQI as an improved means for an objective modification of milk purchase price and for pressure on further RMQ improvement. The study was performed for further promotion of milk food chain quality and safety as well as for the improvement of the competitive ability of the dairy production branch.

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SOMATIC CELL COUNT IN GOAT MILK

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ABSTRACT

The study monitored somatic cell count (SCC) in raw goat milk in goats from an intensive farm (Farm A) and from a small operation (Farm B) in the period between April 2006 and November of the same year. A total of 42 bulk tank samples of milk were collected from Farm A and compared with 15 samples from Farm B, both farms situated in the Czech Republic. All samples were processed by a fluoro-opto-electronic method.

In addition to that the dynamics of total micro-organisms and *Staphylococcus aureus* counts were monitored for the benefit of comparison with SCC.

The mean SCC on Farm A was 1875 ± 476.10³ ml⁻¹. The lowest mean SCC was detected in July, namely 1395.10³ ml⁻¹ and the highest in November at 2802.10³ ml⁻¹. The mean SCC on Farm B was 895 ± 112.10³ ml⁻¹. The lowest SCC on this farm at 781.10³ ml⁻¹ was found in October and the highest at 1049.10³ ml⁻¹ in May. Comparison of SCC levels on both farms showed a significantly higher count of SCC in goat milk samples from Farm A.

Key words: fluoro-opto-electronic method; somatic cell count; *Staphylococcus aureus*; White Shorthaired Goat;

INTRODUCTION

Somatic cell count (SCC) in goat milk can be similar or higher than in the cow milk (24), provided that the milk comes from healthy animals (8). Changes in SCC are affected by the method of milking and the health status of the animals.

Higher SCC in goat milk can also be caused by a different type of secretion in goats, namely apocrine secretion, as opposed to the merocrine secretion in cows (22). It is the presence of non-leukotic, cell resembling fragments in goat milk that can increase the overall SCC values. The customary high SCC in goat milk is caused by a higher cytoplasmic particle count originating in apocrine secretion in the mammary gland. Furthermore, also the method of SCC determination takes its toll (25).

Somatic cells are also indicative of mastitis. Mastitis has an impact on the economics of milk production, decreases its quality and technological properties. Along with mastitis, also the risk of pathogenic macro-organism and antibiotic residuals in milk increases. Somatic cells comprise more cell types: polymorphonuclear leukocytes, macrophages, lymphocytes and epithelial cells. The above mentioned polymorphonuclear leukocytes form more than 40 % of the overall SCC, especially in goat milk, where total SCC exceed 1.10^6 in 1 ml (10). Somatic cell counts increase in the initial stage of the mammary gland inflammation and drop gradually with treatment. The teat canal represents the first barrier against bacterial infection. Bacteria which pass through this first barrier face another barrier in the milk cistern in the form of phagocytes leukocytes. The main task of neutrophilic leukocytes is to eliminate the contagion by means of phagocytes. An increase in somatic cell count is a normal physiological response of the organism to the infection (25). That is to say that increased SCC values announce the compromising of milk quality and thus bring along its exclusion from human consumption (15). Gajdůšek *et al.* (11) monitored the properties and composition of goat milk and found that SCC values ranged from 78.10^3 ml⁻¹ to 4520.10^3 ml⁻¹. They demonstrated highly negative relations between SCC and

the lactose content, casein count, titrable acidity, chlorine-sugar and alcohol scores. Furthermore, they demonstrated highly negative relations towards the N-test values, mineral content, milk conductivity and rennetability. They found that SCC values in goat milk exceeding 175.10^3 ml^{-1} were accompanied by decrease in fat content and, on the opposite, increase in protein content.

Hygiene criteria of goat milk quality are laid down by Commission Regulation No. 166/2006 (1). No limit has been laid down for goat milk SCC. The Regulation only lays down a limit on plate count (PC). The PC standard per ml for raw goat's milk at 30 °C is ≤ 1 500 000 if it is intended for the manufacture of heat-treated, milk-based products and ≤ 500 000 if it is intended for the manufacture of products made with raw milk by a process which does not involve any heat treatment and it is expressed as a geometric mean over a period of two months, with at least two samples a month.

The aim of the present study was to compare the effect of SCC, method of breeding and effect of milking on two farms with different size of herd.

MATERIAL AND METHODS

Goat milk samples were collected from two farms situated in identical climatic conditions. On the Farm A there were 75 goats of White Shorthaired breed between the 1st and 8th lactation. The mean daily milk yield was 2—3 kg of milk, the mean annual milk yield was 600—800 kg of milk. In the period between mid-May and mid-November the goats had access to pasture *ad libitum* and were provided 0.5 kg of hay and 1 kg of grain cereals, vitamin and mineral mixture and salt blocks for licking. In the winter, the feed rations contained 2—3 kg of fodder silage, 1 kg beet silage, 1 kg hay and a maximum of 1 kg of grain cereals, vitamin and mineral mixture and salt blocks for licking. They were milked twice a day by milking machine. Milk samples were collected after weaning of kids in the course of lactation between the end of April and the beginning of November 2006 in regular time intervals. On the whole, 42 bulk tank samples of raw milk were collected. The average milk composition and physical and chemical properties were as follows: protein 2.78 ± 0.225 %, fat 3.06 ± 0.308 %, lactose 4.52 ± 0.043 %, fat-free dry matter 7.84 ± 0.215 %, dry matter 11.06 ± 0.515 %, titrable acidity 5.54 ± 0.683 °SH, pH 6.49 ± 0.406, freezing point -0.550 ± 0.004 °C, rennetability 93.33 ± 14.76 s, conductivity 7.36 ± 0.205 mS.cm^{-1}.

Farm B bred 7 goats of White Shorthaired breed between the 1st and 5th lactation, with a mean daily milk yield of 1—1.5 kg, i.e. 400—500 kg per year. The goats were fed summer diet, involving mostly pasture, and winter diet involving mostly fodder silage, hay and potatoes. They were milked manually twice a day. The average milk composition and physical and chemical properties were as follows: protein 3.16 ± 0.162 %, fat 3.72 ± 0.665 %, lactose 4.78 ± 0.068 %, fat-free dry matter 8.51 ± 0.217 %, dry matter 12.35 ± 0.634 %, titrable acidity 6.75 ± 0.464 °SH, pH 6.06 ± 0.942, freezing point -0.560 ± 0.012 °C, rennetability 55.63 ± 0.884 s, conductivity 6.75 ± 0.606 mS.cm^{-1}. On the whole, 15 samples were collected and examined for the benefit of comparison. The evaluations took place in the following months: May, August, October and November.

Somatic cell count was established using fluoro-opto-electronic method specified in ČSN EN ISO 13366-3/1998 (6).

Milk samples from the Farm A were also subjected to microbiological examination. Basic sample processing was carried out according to ČSN ISO 7218 1998 (4). The following microbiological indicators were assessed: total number of colonies (3) and *Staphylococcus aureus* count (5).

All animals were in good condition throughout the observation period.

RESULTS AND DISCUSSION

Limit for SCC in cow milk is laid down by Commission Regulation No. 166/2006 at the level of 400.10^3 ml^{-1} (1). However, there is no limit for goat milk. Generally, higher levels of SCC are found in goat milk due to a larger volume of cytoplasmic mass as a consequence of apocrine type of secretion in the goat's mammary gland (13, 19). Contreras (2) contemplated that the reason behind increased somatic cell count can be *Staphylococcus aureus* which is considered the most frequent pathogenic micro-organism in goats. The mean SCC determined

Table 1. Mean SCC[1] (10^3 ml^{-1}) in goat milk on Farm A

	Month								Year 2006
	April	May	June	July	August	September	October	November	
Mean	2219	1396	1726	1395	1540	1948	1971	2802	1875
SD	374	121	1132	387	834	239	262	606	476
min	1841	1217	875	959	1239	1688	1456	2244	1395
max	2695	1528	3010	1700	2095	2327	2398	3456	2802

[1] – Somatic cell count; SD – standard deviation

Table 2. Mean SCC[1] (10^3 ml^{-1}) in goat milk on Farm B

	Month				Year 2006
	May	August	October	November	
Mean	1049	881	781	870	895
SD	570	424	433	870	112
min	286	396	226	321	781
max	1667	1179	1209	2191	1049

[1] – Somatic cell count; SD – standard deviation

Table 3. Mean count of *Staphylococcus aureus* (CFU.ml^{-1}) in goat milk on Farm A

	Month							
	April	May	June	July	August	Sept.	October	November
Total number of colonies	6.6×10^{4}	3.2×10^{4}	9.0×10^{4}	2.1×10^{5}	2.4×10^{5}	1.2×10^{5}	1.3×10^{5}	6.0×10^{4}
S. aureus	6.0×10^{2}	7.3×10^{1}	1.1×10^{2}	3.3×10^{1}	1.5×10^{1}	$< 5.0 \times 10^{1}$	1.6×10^{2}	2.4×10^{2}

Table 4. Mean count of *Staphylococcus aureus* (CFU.ml^{-1}) in goat milk on Farm B

	Month			
	May	June	October	November
Total number of colonies	2.7×10^{4}	3.5×10^{4}	6.2×10^{4}	3.1×10^{4}
S. aureus	2.0×10^{1}	0	0	1.0×10^{1}

on Farm A was 1875 ± 476.10^{3} ml^{-1} (Tab. 1). This value was significantly higher than $1274.4.10^{3}$ ml^{-1}, the value reported by Pernthaner *et al.* (20). We detected the lowest SCC in May, at the level of 1396 ± 387.10^{3} ml^{-1}. Sheldrake *et al.* (23) detected the lowest SCC at the start of lactation at 438.10^{3} ml^{-1}and the highest in August and September at 1684.10^{3} ml^{-1}. Our findings indicated the highest count in November, at 2802 ± 606.10^{3} ml^{-1}. We also detected a high SCC at the start of lactation in April at 2219 ± 10^{3} ml^{-1} which could have been caused by the mechanical damage to the mammary glands caused by the kids (9). On the contrary, Gomes *et al.* (12) detected a growing tendency in SCC in the second half of lactation.

The mean SCC in milk from Farm B was 895 ± 112.10^{3} ml^{-1} (Tab. 2). It was a value which approached the value of 800.10^{3} ml^{-1} presented by Zadražil (27). The lowest SCC of 781 ± 433.10^{3} ml^{-1} was found in October and the highest of 1049 ± 570.10^{3} ml^{-1} in May. The results established in longer intervals than in the case of milk from Farm A indicated that SCC values are balanced, which is what can be expected in the course of the whole lactation period. Comparison of SCC determined in goat milk samples from both farms (Fig. 1) allow us to conclude that SCC in milk from Farm A was significantly higher ($P \leq 0.01$). We assume that the differences were caused by different hygiene habits and more friendly milking attitude of staff in small operations.

Table 3 shows that the total number of colonies (TNC) in goat milk from Farm A ranged between 10^{4} and 10^{5} CFU.ml^{-1} ($3.2\ .10^{4}$–$2.1.10^{5}$ CFU.ml^{-1}) throughout the monitored period. TNC in goat milk from Farm B (Tab. 4) ranged between $2.7\ .\ 10^{4}$ and $6.2\ .\ 10^{4}$ CFU.ml^{-1}). By confronting the TNC dynamics and SCC in the individual months, no correlation has been found between the SCC change and the TNC change on both Farms. For raw goat milk, there is a limit set by Commission Regulation No. 166/2006 (1) as a rolling geometric average of the TNC at $\leq 1.5\ .\ 10^{6}$ CFU.ml^{-1}. Figure 2 makes it clear that this limit was not exceeded throughout the period of evaluation. The issues related to the total number of colonies. The relationship between total number of

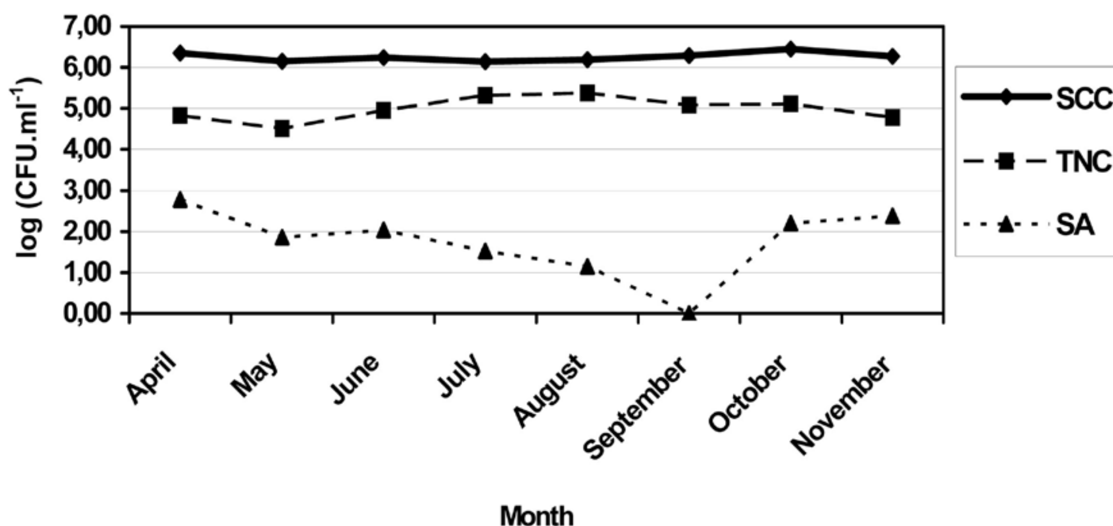

Fig. 1. Dynamics of SCC and the monitored microbiological indicators in goat milk from Farm A
SCC – somatic cell count; TNC – total number of colonies; SA – *Staphylococcus aureus*

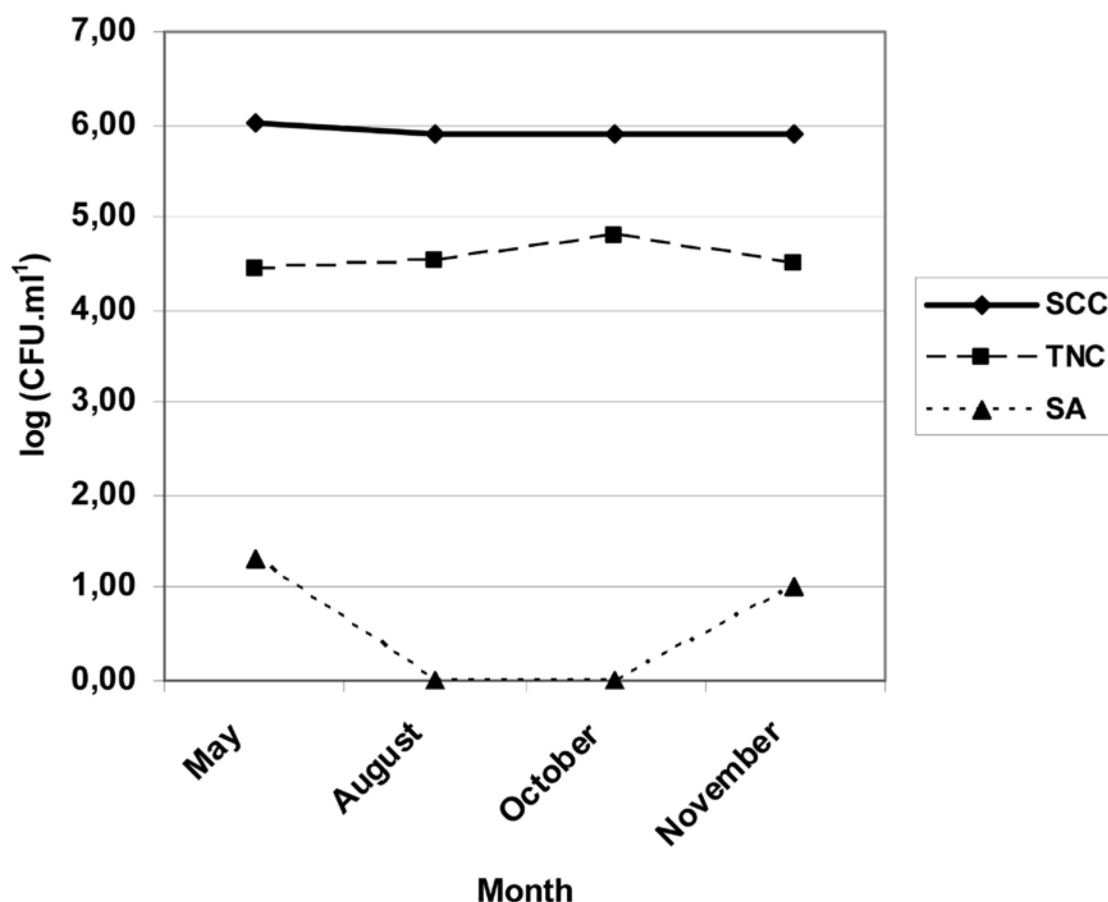

Fig 2. Dynamics of SCC and the monitored microbiological indicators in goat milk from Farm B
SCC — somatic cell count; TNC — total number of colonies; SA — *Staphylococcus aureus*

colonies and somatic cell count in raw goat milk was investigated Ying *et al.* (26) who did not establish correlation between these two indicators.

Staphylococcus aureus count on Farm A ranged approximately between 10^1—10^2 CFU.ml^{-1} (1.5 . 10^1—6 . 10^2 CFU.ml^{-1}) with the exception of September when *S. aureus* was not detected in milk (Fig 1). Over the majority of the monitored period, *S. aureus* count changes corresponded to the dynamics of SCC (Fig. 2). *S. aureus* count on Farm B ranged approximately between 1.0 . 10^1 and 2.0 . 10^1 CFU.ml^{-1} with the exception of August and October when *S. aureus* was not detected (Tab. 4).

One of the factors which significantly partake in the increased somatic cell count in raw goat milk is subclinically ongoing mammary gland inflammation (16, 21). Leitner *et al.* (14) as well as Moroni *et al.* (17) agreed in their conclusions that the most frequent cause of mammary gland inflammation in goats were group coagulase-negative staphylococci, especially the *Staphylococcus epidermidis* and *Staphylococcus caprae* species. *Staphylococcus aureus* was mentioned as the second most frequent agent. On the other hand, Deinhofer and Pernthaner (7) identified *S. aureus* as the main cause of mastitis in goats. *S. aureus* infection can bring about an increase in somatic cell counts as high as 1.5 of the logarithmic order (18). Moroni *et al.* (18) reported that intramammary infection was diagnosed in the cases of raw milk *S. aureus* occurrence at ≥ 100 CFU.ml^{-1}.

No significant differences (P = 0.05) in *S. aureus* counts were found between the Farm A and Farm B. Statistically significant differences (P = 0.05) were detected in TNC and SCC.

CONCLUSION

The study monitored somatic cell count (SCC) in raw goat milk from an intensive farming herd (Farm A) and from a small operation (Farm B). The mean SCC on Farm A was 1875 ± 476 . 10^3 ml^{-1} (1395 . 10^3 ml^{-1}—2802 . 10^3 ml^{-1}) and on Farm B it reached 895 ± 112 . 10^3 ml^{-1} (781 . 10^3 ml^{-1}—1049 . 10^3 ml^{-1}). Comparison of the SCC values on both farms allowed us to conclude that a significantly higher count was determined in goat milk samples from Farm A, which acknowledges the influence of the method of breeding and milking. The basic microbiological examination and monitoring of the relevant parameters (TNC, SA, SCC) detected higher incidence of *S. aureus* at the beginning (April) and the end of lactation (October, November) on both farms in the

course of our study which corresponded to the highest measured somatic cell count levels. It can be concluded that contrary to the total micro-organism count, there is a correlation between *S. aureus* count and the somatic cell count in raw goat milk.

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TETRACYCLINE RESISTANCE OF STAPHYLOCOCCI ISOLATED FROM GAME MEAT AND MECHANICALLY DEBONED POULTRY MEAT

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ABSTRACT

Susceptibility to tetracyclines has been tested in three groups of staphylococcal isolates. The first group included 18 coagulase-negative staphylococci isolated from brown hares, the second group 9 coagulase-negative and 18 coagulase-positive staphylococcal isolates from pheasants and the third group 17 coagulase-negative and 15 coagulase-positive strains isolated from mechanically deboned poultry meat. The resistance achieved 29 % in brown hares, 26 % in pheasants and 22 % in mechanically deboned poultry meat. The minimum inhibitory concentrations (MICs) of tetracycline ranged from 0.5 mg.l^{-1} to 64 mg.l^{-1} depending on the susceptibility of individual isolates to tetracyclines. Three coagulase-negative isolates from brown hares and five isolates (two coagulase-negative and three coagaluse-positive) from pheasants were not inhibited even with the highest tetracycline concentration tested (64 mg.l^{-1}).

Key words: game meat; poultry meat; resistance; staphylococci; tetracyclines

INTRODUCTION

The development of microbial resistance is an increased problem worldwide. However, this problem has several dimensions and it is partially associated with an increased antibiotic consumption. The prevalence of resistance to some antibiotics has been directly proportional to antibiotic consumption (chinolons in Spain, macrolides in Italy) but also negative correlations were described (12). Therefore, it is incorrect to connect a high prevalence of resistance explicitly with a higher antibiotic consumption because various factors actively participate in the development of resistance.

Although the type of host, bacterial species or individual antibiotic are important factors in spreading resistance, also gene mutations induced by the presence of an antibiotic in the environment, mobile genetic transfers, clonal disseminations, local antibiotic policy, presence of schemes and directives in regard to antibiotics, reimbursement of the cost of antibiotics or availability and the quality of the health care system have to be taken into account (12).

Food chain is considered as a potential hazard in transmission of antibiotic resistant bacteria between animal and human population (13). Antibiotic-resistant strains can be consumed by humans and become a source of genes encoding antibiotic resistance for bacteria present in the digestive tract.

Prevalence of infections caused by various bacterial species and strains has changed and in the last twenty five years the importance of gram-positive bacteria has been reported worldwide (19), the most discussed bacteria being *Staphylococcus aureus*, coagulase-negative staphylococci and enterococci. Currently, medical attention is paid mainly to coagulase-negative staphylococci because they represent a serious therapeutic problem. However, they are generally considered as almost non-pathogenic micro-organisms. Coagulase-negative staphylococci play a major role as an activator of nosocomial infections and they often become multiresistant to antibiotics (3, 4, 9).

In this study, the occurrence of staphylococci resistant to tetracyclines isolated from game meat and mechanically deboned poultry meat as well as their possible role in the transmission of antibiotic resistance were reported.

MATERIAL AND METHODS

Sampling and preparation of samples for microbiological examination were performed in accordance with STN ISO 3100-2 (16). Staphylococci were isolated from abdominal muscles of 8 brown hares (*Lepus europeus*) hunted in the region Wildendürnbach (north-east Austria), and from thigh muscles of 8 pheasants (*Phasianus colchicus*) bred on Wild and Game Animal Breeding Farm and Hunting Area in Rozhanovce, as well as from 15 samples of mechanically deboned poultry meat produced in poultry-processing plant in Košice (eastern Slovakia) and divided into three groups according to their origin. The first group included 18 coagulase-negative staphylococci isolated from brown hares, the second group 9 coagulase-negative and 18 coagulase-positive staphylococcal strains isolated from pheasants and the third group contained 17 coagulase-negative and 15 coagulase-positive staphylococci isolated from samples of mechanically deboned poultry meat.

Stock suspension and appropriate decimal dilutions of individual samples were prepared by a standard procedure according to STN ISO 6887 (17). The first two decimal dilutions (10^{-1} and 10^{-2}) were used for determination and enumeration of coagulase-positive staphylococci in accordance with requirements of STN EN ISO 6888-1 using Baird-Parker agar medium (HiMedia, India) (18). After 48 hours of incubation at 37 °C, Petri dishes with 15—150 typical or atypical colonies were selected for further investigation. Bacterial suspensions were prepared in BHI (Brain Heart Infusion) broth (Oxoid, Great Britain). Each inoculum was adjusted to match the 0.5—1 McFarland turbidity standard and the test tubes were incubated for 24 hours at 37 °C. The coagulase test was carried out with Staphylo PK test (Imuna Šarišské Michaľany, Slovakia) in accordance with the instructions of the producer. The evaluation was performed after 1, 2, 3, 6 and 24 hours of incubation.

Staphylococcal susceptibility to tetracyclines was determined with the help of disc diffusion (5) and agar dilution methods (6).

Disc diffusion method: The bacterial inoculum (0.1 ml) adjusted to the 0.5—1 McFarland standard was uniformly spread over the surface of a sterile Mueller-Hinton agar (HiMedia, India) with a sterile bent rod. Paper discs (Oxoid, Great Britain) impregnated with specific concentration of tetracyclines (TE 30 μg) were placed on the surface of the medium. The plates were incubated 24 hours at 37 °C and diameters of the zones of inhibition (including the disc) were measured in milimetres (mm).

Agar dilution method: Mueller-Hinton agar (HiMedia, India) was used for the detection of minimum inhibitory concentrations (MICs). The drops (0.5 μl) of 24-hour suspension of staphylococcal strains tested (adjusted to the 0.5—1 McFarland turbidity standard) were placed parallel to each other onto the surface of control plates and plates with the agar containing the following concentrations of tetracyclines: 0.5; 1.0; 2.0; 4.0; 8.0; 16.0; 32.0; 64.0 $mg.l^{-1}$. The inoculated plates were incubated at 37 °C for 24 hours and then the lowest concentration of tetracycline preventing growth of macroscopically visible colonies of staphylococci was determined.

All staphylococci strains were classified according to the zone diameter interpretative standards and equivalent minimal inhibitory concentration breakpoints for *Staphylococcus* spp. established by CLSI document M100-S16 (7) as follows: resistant (zone of inhibition ≤ 14 mm and MIC ≥ 16 $mg.l^{-1}$), intermediate (zone of inhibition = 15—18 mm and MIC = 8 $mg.l^{-1}$) and susceptible (zone of inhibition ≥ 19 mm and MIC ≤ 4 $mg.l^{-1}$).

RESULTS

Based upon the results in Table 1, which were compared with the interpretative criteria for agar dilution ($mg.l^{-1}$) and disc diffusion method (mm) set by CLSI (7), twelve isolates from brown hares (Group 1) were classified as susceptible and five strains as resistant to tetracyclines. One strain (No. 15) could neither be classified as susceptible nor as intermediate because results obtained by both methods did not correspond.

Four coagulase-negative and three coagulase-positive staphylococcal isolates from pheasants (Group 2) were classified as resistant to tetracyclines. Similar results were also obtained in mechanically deboned poultry meat (Group 3) where five coagulase-negative and two coagulase-positive strains were identified as resistant to tetracyclines.

The proportion of resistant staphylococcal isolates from brown hares achieved 29 %, in pheasants 26 % and in mechanically deboned poultry meat 22 % (Tab. 2). MICs of tetracycline in all strains tested ranged from 0.5 $mg.l^{-1}$ to 64 $mg.l^{-1}$ on dependance of their susceptibility to tetracyclines. The growth of three coagulase-negative staphylococci isolated from brown hares and five strains (2 coagulase-negative and 3 coagaluse-positive) isolated from pheasants was not inhibited even with tetracycline concentration of 64 $mg.l^{-1}$. Thus, the MICs of tetracycline in these isolates will probaly be higher than 64 $mg.l^{-1}$.

Considering the results of both the disc diffusion and agar dilution methods, the resistance to tetracyclines was definitely confirmed in 19 isolates of staphylococci from game meat and mechanically deboned poultry meat out of 77 strains tested (25 %) (Tab. 2).

DISCUSSION

During the 1950s and 1960s, tetracycline was one of the most widely used antibiotics in human and veterinary medicine. Oxytetracycline was also widely used as an additive to livestock feed because it stimulated weight gain in some domestic animals. It has been fed routinely to calves, chickens, turkeys, sheep, and pigs. Tetracycline has also been used to improve the health and promote the growth of fish in commercial fisheries. These uses of tetracycline have been controversial because of fears that such widespread nonclinical use would increase the incidence of tetracycline resistant strains (8). However, this fact is also typical for other antibiotics used in human and veterinary medicine.

Generally, the occurrence of resistant *Staphyloccoccus aureus* and coagulase-negative staphylococci isolated from

Table 1. The resistance of staphylococcal isolates to tetracycline

Group I (brown hares)					Group II (pheasants)					Group III (mechanically deboned poultry meat)									
No	**coa-gulase**	**MIC mg.l^{-1}**	**ZI Mm**		**No**	**coa-gulase**	**MIC mg.l^{-1}**	**ZI mm**		**No**	**coa-gulase**	**MIC mg.l^{-1}**	**ZI mm**		**No**	**coa-gulase**	**MIC mg.l^{-1}**	**ZI mm**	
1	CN	4	31	**S**	1	CN	0.5	20	**S**	1	CN	0.5	26	**S**	28	**CP**	0.5	34	**S**
2	CN	0.5	30	**S**	2	CN	0.5	30	**S**	2	**CP**	64	10	**R**	29	**CP**	1	26	**S**
3	CN	1	22	**S**	3	**CP**	0.5	34	**S**	3	CN	0.5	35	**S**	30	CN	64	12	**R**
4	CN	1	20	**S**	4	**CP**	0.5	30	**S**	4	CN	1	33	**S**	31	**CP**	1	20.5	**S**
5	CN	4	20	**S**	5	CN	8	17	**S**	5	**CP**	1	35	**S**	32	CN	0.5	35	**S**
6	CN	2	22	**S**	6	CN	0.5	35	**S**	6	CN	64	12	**R**					
7	CN	> 64	0	**R**	7	**CP**	4	24	**S**	7	**CP**	64	10	**R**					
8	CN	> 64	0	**R**	8	CN	32	12	**R**	8	**CP**	64	10	**R**					
9	CN	2	22	**S**	9	**CP**	0.5	24	**S**	9	CN	1	30	**S**					
10	CN	> 64	8	**R**	10	**CP**	0.5	25	**S**	10	**CP**	64	9	**R**					
11	CN	1	20	**S**	11	**CP**	0.5	25	**S**	11	**CP**	64	13	**R**					
12	CN	16	14	**R**	12	**CP**	> 64	10	**R**	12	CN	2	29	**S**					
13	CN	2	21	**S**	13	**CP**	> 64	7	**R**	13	CN	1	19	**S**					
14	CN	0.5	24	**S**	14	CN	> 64	0	**R**	14	**CP**	0.5	29	**S**					
15	CN	8 I	21 S		15	**CP**	> 64	0	**R**	15	**CP**	1	30	**S**					
16	CN	2	20	**S**	16	**CP**	1	44	**S**	16	CN	1	33	**S**					
17	CN	32	8	**R**	17	**CP**	1	34	**S**	17	CN	1	32	**S**					
18	CN	2	26	**S**	18	CN	0.5	26	**S**	18	**CP**	0.5	23	**S**					
					19	CN	2	28	**S**	19	**CP**	1	26	**S**					
					20	**CP**	32	10	**R**	20	**CP**	1	25	**S**					
					21	**CP**	1	32	**S**	21	CN	1	25	**S**					
					22	**CP**	0.5	26	**S**	22	CN	1	25	**S**					
					23	**CP**	1	28	**S**	23	CN	1	29	**S**					
					24	**CP**	0.5	26	**S**	24	CN	1	23	**S**					
					25	**CP**	0.5	28	**S**	25	**CP**	1	26	**S**					
					26	CN	> 64	10	**R**	26	CN	1	23	**S**					
					27	**CP**	2	24	**S**	27	CN	1	24	**S**					

CN – coagulase-negative, CP – coagulase-positive, R – resistance, I – intermediate susceptibility, S – susceptibility, MIC – minimal inhibitory concentration, ZI – zone of inhibition

Table 2. Total percentage of resistance to tetracycline of staphylococcal isolates from hares, pheasants and mechanically deboned poultry meat

	Group 1 (n = 18)		**Group 2 (n = 27)**		**Group 3 (n = 32)**		**Total (n = 77)**	
	CN (n = 18)	CP (n = 0)	CN (n = 9)	CP (n = 18)	CN (n = 17)	CP (n = 15)	CN (n = 44)	CP (n = 33)
R	5 (29)	0	3 (33)	4 (22)	2 (12)	5 (33)	**19 (25)**	
	(29)		(26)		(22)			
S	12 (71)	0	6 (67)	14 (76)	15 (88)	10 (67)	**57 (75)**	
	(71)		(74)		(88)			

CN – coagulase-negative, CP – coagulase-positive, R – resistance,
S – susceptibility, number depicted in the parenthesis is percentage

food as milk, meat, and poultry to selected antibiotics (including tetracyclines) permanently increases.

Two hundred and sixteen isolates of *Staphylococcus aureus* were obtained from raw milk within different locations in the Rift Valley of Kenya by Shitandi and Mwangi (15). Their resistance profiles to six families of antimicrobials were evaluated using the plate diffusion method. Resistance to penicillin (72.2 %) was the most frequent followed by that to trimethoprim + sulfamethazin (59.2 %); tetracycline (57.9 %); erythromycin (21.3 %); chloramphenicol (46.8 %) and methicillin (7.8 %).

Similar results were obtained by Meaney and Flynn (14) in Ireland using the disc diffusion method. Resistance to penicillin was confirmed in 65 %, to tetracycline in 29 % and to erythromycin in 17 % staphylococcal isolates.

Based on data of the Hungarian resistance monitoring system performed in 2001, the antibiotic resistance of *Staphylococcus* strains of human and animal origin was studied by Kaszanyitzky *et al.* (10). The most frequent antibiotic resistance profiles of strains isolated from animals and food were penicillin/tetracycline, penicillin/lincomycin and penicillin/lincomycin/tetracycline. Penicillin/tetracycline resistance was exhibited by strains from samples of the meat industry, poultry flock, poultry industry, noodle and horses.

As reported, the occurrence of resistant strains in producing animals is a bit higher than that being isolated from food of animal origin (1, 2, 11).

As follows from results of this study, the proportion of staphylococci isolates resistant to tetracyclines was 29 % in brown hares, 26 % in pheasants and 22 % in mechanically deboned poultry meat. The highest level of tetracycline resistance was surprisingly detected in coagulase-negative isolates from brown hares in spite of the fact, that those free-living animals were most likely not in a contact with any antibiotic. This may be probably explained by a horizontal transfer of antibiotic-resistance gene from the resistant bacterium to another bacterium normally susceptible to this antibiotic.

As seen from the results, tetracycline resistant coagulase-negative staphylococci can be isolated from the game (brown hares, pheasants) and of mechanically deboned poultry meat. Due to its significant increasing the microbial resistance to antimicrobial substances has become an alarming factor. An important way how to solve the world-wide problem of development and spread of the microbial resistance to antibiotics is its monitoring combined with other measurements (e. g. rationalisation of antibiotic prescription and other measures).

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DISCUSSION

Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results sections. Include in the Discussion section the implications of the findings and the limitations, together with their significance for future research. Relate the observations to other relevant studies.

CONCLUSIONS

Link the conclusions with the aims of the study, but avoid unqualified statements and conclusions not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. Recommendations, when appropriate, may be included.

ACKNOWLEDGEMENT

(in italics) Those who have given technical assistance, or moral or financial support, or supplied equipment or materials, or engaged in translation or general supervision, etc., should be recognized in the Acknowledgements.

REFERENCES

As described above.

Notes and Short Communications. Such manuscripts should have the same form as full papers, but are much shorter. Separate headings are needed only for the Abstract, Key words, Main Text, Acknowledgements and References. These scripts fall under the above main headings and should be marked accordingly.

Technical Notes. Such notes should record a new method, technique, or procedure of interest to veterinary scientists. They should include the reason(s) for the new procedure, a comparison of results obtained by the new method with those from other methods, together with a discussion of the advantages and disadvantages of the new technique. A technical note should not exceed six pages, including figures and tables.

Research Communications. These are short articles, no more than four pages, which should introduce novel and significant findings to the commonwealth of veterinarians.

Review Articles. These should provide a substantial survey, with an appropriate historical perspective, of the literature on some aspect of veterinary medicine. Alternatively, such articles may review a topic of veterinary interest, which may not come within the normal purview of many veterinarians. Authors submitting review manuscripts should include a section describing the methods used for locating, selecting, extracting, and synthesizing data. These methods should be summarized in the abstract.

Observations. Research of this kind contributes to knowledge, but not to the advancement of ideas or the development of concepts. In some cases, these papers underpin what may seem obvious, with statistical data. Such communications should not exceed four pages.

Current Issues. Papers that deal with issues of topical interest to veterinary scientists will be considered. Issues may include items on environmental concerns, legislative proposals, etc.

Book reviews. Book reviews may be submitted. They should bring a new text to the readership and evaluate it.

Letters to the Editor. These are items of scientific correspondence, designed to offer readers the chance to discuss or comment on published material and for authors to advance new ideas. Should a letter be polemical, a reply or replies for simultaneous publication may be sought from interested parties.

Editorial Board