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LATE ABSTRACTS

DETECTION OF BOVINE LEUKEMIA VIRUS PROVIRAL DNA IN YAROSLAVSL, MONGOLIAN AND BLACK PIED CATTLE BY PCR

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INTRODUCTION

The nucleotide sequence of bovine leukemia virus (BLV) is approximately 50% similar to that of human T lymphocyte virus (HTLV) types I and II [1]. These viruses have common regulatory mechanisms [2] but differ in host cell tropism, with B cells comprising the primary target of BLV infection [3]. BLV is mainly transmitted horizontally by direct exposure to biological fluids (blood, milk, saliva and semen) contaminated with infected lymphocytes. Several authors have shown that it is possible to establish BLV free herds by identifying seropositive animals and eliminating them from the herds (e.g. see [4]). Others demonstrated that serological test is not sensitive enough to find all BLV infected cattle [5]. In the infected cells, BLV is integrated into host DNA in the form of provirus which can be detected by different molecular biology methods. Recently, polymerase chain reaction (PCR) has been described for the detection of BLV proviral DNA [6]. PCR enhances the specificity and sensitivity of BLV detection [7].

Here we report the application of PCR for detection of the proviral BLV DNA in Yaroslavl, Mongolian and Black Pied cattle for the first time. These breeds of cattle were selected due to the fact that they possess a series of valuable traits that discriminate them as unique breeds.

MATERIALS AND METHODS

In this study blood samples from a total number of 205 cows comprising of 120 Yaroslavl breed, 50 Mongolian breed and 35 Black Pied breed were collected (6ml from each animal). Wersen II was used as an anticoagulant and DNA was isolated from aliquots of 200 μ l of the blood by DiatomTM DNA Prep 200 kit (IsoGene, Russia). Total DNA concentration was determined by agarose/ethidium bromide gel electrophoresis by comparison with phage DNA lambda. The PCR was performed in a total reaction volume of 25 μ l employing

about 100-150 ng of DNA followed by viral gene detection using a GenePak™ DNA PCR test kit (IsoGene, Russia). For PCR two pairs of primers with the following sequences were employed: gag 1: 5' GGAGGWGGRA AGATGCGAACTATT 3' and gag 2: 5' GTCCGYTCTACYAACCC TGAA CTT 3' (347 bp fragments of BLV gene) as well as pol 1: 5' GAGGTTT GTGCATGAYCTACGAGYTACA 3' and pol 2: 5' TAGAGACCCAYTGGA GGTCTCCYAAGAC 3' (599 bp fragments of BLV gene). The PCR thermal profile was as follows: the first cycle of denaturation at 95°C for 60s, annealing primers at 62°C for 40s and elongation at 74°C for 90s. The second cycle of denaturation at 95°C for 30s, annealing primers at 60°C for 30s, and elongation at 74°C for 60s. 43 cycles of denaturation at 95°C for 20s, annealing primers at 58°C for 20s, and elongation at 74°C for 40s. After the last cycle the tubes were incubated at 74°C for 60s.

RESULTS AND DISCUSSION

BLV infection is endemic in some countries. In Russia the problem of prevention and eradication of BLV and other viral infections among farm animals remains a major concern despite all measures. In the United States alone, the estimated loss to the dairy industry because of BLV infection is reported to be more than \$86 million annually [8]. Recently, 60% positive results were obtained by PCR among the cows belonging to Polish Black-and-White breed [7]. The detection of BLV infection in cattle is carried out by different methods including syncytial test, serological tests and PCR. It is known that the PCR technique is a more sensitive and more accurate BLV diagnosis method. On the other hand, many groups including Limansky et al [9] have shown that the accuracy and reliability of PCR analysis depend on the primer type.

In the present study, among the 120 animals belonging to Yaroslavl breed 21 positive and 7 doubtful results in the first PCR (fragment of BLV gene was 347 bp) and 0 positive and 0 doubtful results in the second PCR (fragment of BLV gene was 599 bp) were obtained. Among the 50 animals belonging to Mongolian breed 4 positive and 1 doubtful results in the first PCR and 0 positive and 0 doubtful results in the second PCR were obtained. Among the 35 animals belonging to Black Pied breed 6 positive and 2 doubtful results in the first PCR and 5 positive and 0 doubtful results in the second PCR were obtained.

Results of the present work indicated that utilizing the second pair of primers (i.e. 599 bp fragments of BLV gene) provide a more accurate viral detection with no doubtful results compared with the first pair of primers (i.e. 347 bp fragments of BLV gene).

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DISTRIBUTION OF DIVALENT ZINC AND COPPER IONS IN THE RAT BRAIN SYNAPTOSOMES: INFLUENCE OF CALCIUM DEFICIENCY AND DEPolarISATION

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INTRODUCTION

The divalent zinc (Zn^{2+}) and copper (Cu^{2+}) ions play fundamental roles in the biochemistry of the human nervous system. In addition, they are regarded as essential trace metals, which are present in high concentrations in brain hippocampus. It is believed that hyperactivity, attention deficit disorder, behaviour disorders and depression are associated with elevated copper and depressed zinc levels. Most patients with autism and paranoid schizophrenia have elevated blood copper levels in addition to other biochemical imbalances. Variations in the levels of Zn^{2+} and Cu^{2+} ions is also believed to be linked to some nervous system disorders such as Alzheimer's disease, Parkinson disease, Wilson's disease, Pick's disease and epileptic seizures [1, 2]. Recent studies have shown that the basic event in Alzheimer's disease is degeneration of neurons due to the accumulation of neurotoxic β amyloid peptides. The accumulation of these peptides leads to an increased intracellular calcium levels, which cause further damage to neurons [3]. High level of Zn^{2+} in Alzheimer's patients may be related to the ability of this ion to accelerate the accumulation of β amyloid peptides [4]. In this study, the concentration of Zn^{2+} and Cu^{2+} ions in the synaptosomes of different areas of rat brain was measured in depolarised conditions. Since there is a close relationship between neural cell function and presence of calcium along with the release of Zn^{2+} and Cu^{2+} ions in synaptic spaces, the amount of these ions in a calcium deficiency condition was also evaluated.

MATERIALS AND METHODS

All the chemicals were of high purity grade and purchased from Merck Co. (Germany). A total number of 45 male Wistar rats (180-230 g) were used in the present study. Animals were handled according to internationally accepted principles for care of laboratory animals. After killing the animals their brains were removed immediately and kept on ice. Brains were then separated into five different regions of cerebellum, hypothalamus, midbrain, striatum and cortex. Slices of these regions were then prepared and homogenised in 0.32 M sucrose and the synaptosomes were prepared according to a procedure explained

previously [5]. The prepared synaptosomes were incubated with phosphate buffer (pH: 7.3, control) or 55 mM potassium ion (depolarisation state) or EGTA (as calcium chelator) for 15 min at 37°C. At end of the incubation period the synaptosomes were burnt to ashes and the remaining Zn^{2+} and Cu^{2+} were measured by atomic absorption spectrophotometry (Perkin-Elmer 2380, USA). The results were analysed by student's t test and $p < 0.05$ was considered significant.

RESULTS

The effect of depolarisation

It was detected that in the presence of potassium ion (depolarisation effect) the concentrations of copper remaining in the synaptosomes of midbrain and cortex are higher than that of the controls. This is while Cu^{2+} levels in the cerebellum, hypothalamus and striatum were lower compared with that of the controls. The differences in the Cu^{2+} levels were statistically significant in the midbrain ($p < 0.01$), cerebellum ($p < 0.01$) and hypothalamus ($p < 0.05$). In the presence of potassium ion higher amounts of Zn^{2+} remained in the cerebellum compared with the cerebellum of controls while it was lower in the hypothalamus, midbrain, striatum and cortex regions.

The effect of calcium ion depletion

In the samples treated with EGTA (calcium ion deficiency) the amount of Cu^{2+} in the cerebellum, hypothalamus and striatum regions were higher, however, the cortex and the midbrain regions showed decreased levels of Cu^{2+} . This is while in the EGTA treated samples Zn^{2+} concentrations were higher in the cerebellum, striatum ($p < 0.05$) and cortex ($p < 0.05$) while it was lower in the midbrain and hypothalamus ($p < 0.001$) regions.

DISCUSSION

Results of the present work showed that there is an intimate relationship between the concentration of divalent metal cations - namely Zn^{2+} and Cu^{2+} - in the neural cells and neural activity. It appears that changes in the equilibrium of these ions can lead to a variety of neural activity disorders. A question to be addressed in a future work is that are the effects of depolarisation and Ca^{2+} deficiency on the Zn^{2+} and Cu^{2+} levels in nerve endings similar in vivo to what we observed here? This question entails further investigation and has the potential in revealing the role of Zn^{2+} and Cu^{2+} in neural transmission and any possible link with other molecules involved in the neurotransmission processes.

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**POSTNATAL HYDROCEPHALIC CSF PROMOTES
DIFFERENTIATION OF CORTICAL PROGENITOR CELLS**

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Our previous work showed that prenatal hydrocephalic CSF at the age of E20 and E21 has an inhibitory effect on the proliferation of cortical progenitor cells and that this inhibitory effect is removed by heating of CSF, suggesting that a protein or peptide is responsible. In this study postnatal hydrocephalic CSF was tested on cortical progenitor cells to investigate whether the inhibitory effect extended beyond the fetal development of the cortex in the rat brain. Normal E18 and E20 fetal cortical cells were cultured in neurobasal media for 24 hours and then affected CSF was added to the cells at 10 and 25% in media. No inhibitory effect was observed at these concentrations, which were effective for prenatal hydrocephalic CSF. Instead, postnatal hydrocephalic CSF was found to promote differentiation giving more processes and branching than the same cells treated with normal postnatal CSF or media alone. Cells treated with affected P2, P4 and P6 CSF all produced more processes and more aggregation of cells than control cells or those exposed to normal CSF. This investigation supports the findings of this lab and others that shunt treatment in neonates for hydrocephalus keeps them alive but does not promote proliferation although differentiation and development of the cortex, with the cells available is promoted. Unlike the finding from studies of prenatal hydrocephalic CSF, heat-inactivation of postnatal hydrocephalic CSF did not suppress the promotion of differentiation suggesting that the factor responsible might be something other than protein or peptide.

In conclusion, it is possible to say that it is vital to remove/block the inhibitory effect of prenatal hydrocephalic CSF before birth since the resulting developmental loss is not recovered by shunting and is no longer present in postnatal CSF.

AN ANALYSIS OF INJURY RISK IN THE MANCHESTER UNITED TEAM WHEN PLAYING AT HOME AND AWAY

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INTRODUCTION

In football, home advantage has been a very important factor in determining the outcome of a game [1, 2]. The results of previous studies have identified four factors thought to be responsible for the home advantage [3]. These can be categorised under the general headings of crowd, learning, travel and rule factors. However, whether home advantage has an effect on the injury or injury risk is not known. This study was aimed at determining the effect on injury risk of playing at home and away, type of playing action, period of the game and zone of the pitch.

METHODS

In order to assess the injury risk for Manchester United (MU) players with respect to (i) playing either at home or away (ii) types of playing action (iii) period of the game and (iv) zone of the pitch, one home (H) and one away (A) game from the F.A. Premier League were chosen for analysis. The study incorporated a hand notation system whereby 16 soccer-specific actions were notated [4]. The injury risk was estimated by subjectively determining if each action had a potential for injury or not and was termed "injury potential". The pitch was divided into 18 zones and the position was recorded along with time elapsed in the game which enabled 15 min periods to be identified. The data were analysed using χ^2 to compare categories, and a level of $P < 0.05$ was used to indicate significance.

RESULTS AND DISCUSSION

Playing either home or away

In the games there was only one injury for a MU player which occurred when playing away. The total number of actions and those with "injury potential" are illustrated in Table 1. The total number of actions for MU (H) was greater than for MU (A), but the difference between them was not significant ($\chi^2 = 2.04$ $P = \text{NS}$). The total number of actions with "injury potential" was significantly greater ($\chi^2 = 24.4$ $P = 0.001$) in MU (A) than MU (H).

Tab. 1. Total number of playing actions and total number of actions with “injury potential” for home and away games.

	Total Actions	Injury Potential
Home Play	1092	244
Away Play	1026	366

Type of playing actions

The total number for each type of action and for those with “injury potential” for home and away is represented in Table 2. A significant difference ($\chi^2 = 63.57$) was found between home and away for each type of action and was generally greater when playing away, particularly those actions involved in contesting the ball.

Tab. 2. Total number for each type of action and the number of actions with “injury potential” for home (H) and away (A) games.

Playing Actions	Total Actions		“Injury Potential”	
	H	A	H	A
Dribbling the ball	12	7	4	3
Goal catch	7	9	5	1
Goal punch	3	5	2	4
Goal throw	6	5	1	2
Heading the ball	69	74	31	55
Jumping to head	52	57	31	44
Kicking the ball	104	99	80	92
Making the tackle	25	36	2	21
Making the charge	5	29	0	5
Passing the ball	378	278	26	21
Receiving the ball	323	257	5	6
Receiving the tackle	36	60	29	41
Receiving the charge	6	33	5	33
Shot on goal	8	12	7	10
Set kick	27	36	14	24
Throw-in the ball	31	29	2	4

Period of the game

A significant difference ($\chi^2 = 15.30$ $P = 0.01$) was found between various periods of the game in relation to actions with “injury potential” (Fig. 1) with noticeably more action with injury potential in the period before half time.

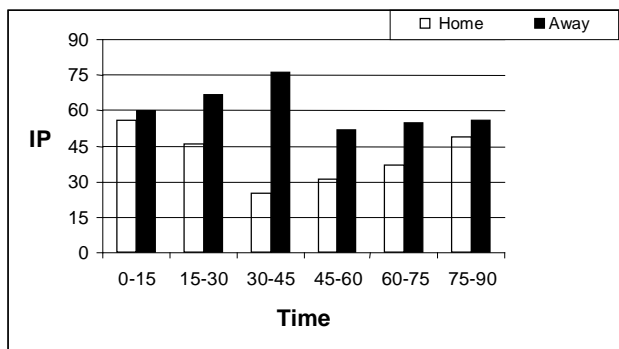


Fig. 1. Total number of actions with “injury potential” (IP) per period (15min each) for home and away games.

CONCLUSIONS

The data suggest that whether playing at home or away does not affect the total number of actions, but it does affect the total number of actions with “injury potential” i.e. the injury risk. When playing away, more of the actions were identified as having injury potential and they were more likely to occur in the later stages of the first half. There was no tendency for injury risk to be related to location on the pitch. The results may be due to the type of game played, but more game would need to be analysed to take this into account. Nevertheless, this approach does give some indication of injury risk and managers, coaches and players should be aware of the possible greater risk of injury when playing away.

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**PATTERN OF EXPRESSION OF THE EUKARYOTIC INITIATION
FACTOR 4E (EIF4E) IN SKIN CANCER**

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Increased protein synthesis is necessary for the transition of cells from quiescence to proliferation. Most of the control of translation occurs at the initiation step. Most eukaryotic mRNAs possess a 5'GpppN "cap" structure, which is recognized by the cap binding protein (eIF4E) as the first step in ribosome scanning. It has been shown that overexpression of eIF4E in NIH 3T3 cells results in transformation and activation of ras oncogene. Suppression of eIF4E activity in transformed cells by overexpression of the eIF4E binding proteins 4E-BP1 or 4E-BP2 results in partial reversal of the transformed phenotype. Elevated eIF4E levels prevent apoptosis in NIH 3T3 cells, whereas overexpression of 4E-BP1 or rapamycin treatment increases the susceptibility to apoptosis. Thus, failure to down-regulate eIF4E may result in loss of growth control. It is clear that eIF4E levels are indeed elevated in a wide variety of transformed cell lines and solid tumors, with the most pronounced increases being observed in breast cancer. The aim of this study was to determine the level of expression of eIF4E in skin cancer, and to ascertain whether or not this factor can be used as diagnostic or prognostic markers within this type of cancer. eIF4E expression increased in most of the skin cancers studied.

We studied tissue samples from 25 patients diagnosed with skin cancer. Bicinchoninic acid method, SDS-PAGE and western blot analysis were used for protein analysis.

The eIF4E levels were significantly higher in tumors compared with normal tissues. Regardless of the mechanism, our study indicates that eIF4E is involved in the development of skin cancer.

ANALYSIS OF MEDICAL ERRORS IN ANAESTHESIA

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The Author is currently doing a major systematic review of studies of medical error causation as part of his PhD project. This report provides general information about the aim and methodology of that review and presents a preliminary result of part of this systematic review as an example of the data analysis and report.

What methods have been used to analyse incidents and medical errors in healthcare, and what are the reported results of the studies.

The review would include any type of empirical studies, quantitative and qualitative from both primary and secondary care. The search strategy included electronic searching of MEDLINE, EMBASE, CINAHL and Psych Info, hand searching of relevant journals, grey literature and conference proceedings and reference checking of the relevant papers. 35457 citations were retrieved from the search, 23605 of them were excluded by checking the titles. From the 11852 remaining citations, about 250 citations seemed to be relevant for the purpose of the whole review. Quantitative studies in English Language, which analysed anaesthesia incidents and were published after 1970, were selected as an example for the purpose of this preliminary analysis.

Reporting of incidents through Critical Incident This was a very commonly used method to analyse anaesthesia incidents. Adverse incidents linked to anaesthesia were reported uncommon and often did not lead to serious patient harm. Most anaesthesia incidents had a multifactorial cause in which human error played a significant part. Human error was reported as the most common sources of mishaps in anaesthesia contributing to 42-82% of incidents. The next important factors included equipment failure and lack of communication following by organizational factors.

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