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Postmortem analysis of the chemical compounds of aqueous humour obtained from the eye of European bison – preliminary results

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Abstract: The aim of this study was the assessment of diagnostic utility of postmortem analysis of aqueous humour in lowland European bison. Samples of aqueous humour were collected from 12 male bison from Białowieża Forest during 24 hours after death. All animals were carefully sectioned and divided into two groups. The first group consisted of young animals from 3 months to 4 years old. Mature individuals 4–16 years old were included in the second group. During 48 hours after collection, all samples were analyzed with colorimetric method. The following parameters were determined: total calcium, ionized calcium, phosphorus, magnesium, sodium and potassium. The concentration of total calcium and ionized calcium as well as calcium/phosphorus and sodium/potassium ratios were higher in group of immature males comparing to fully mature individuals. In contrast, mature males had twofold higher phosphorus level than young animals. Similarly, concentration of magnesium, sodium and potassium were lower in animals belonging to the first group. Biochemical analysis of aqueous humour composition can be an useful diagnostic tool in cause of death determination, and appropriate interpretation of causes of pathological lesions found during necropsy. However, to make the results reliable, first reference values of various chemical compounds of aqueous humour in European bison should be established.

Key words: European bison, eye, biochemistry, aqueous humour

Introduction

The use of aqueous humour for postmortem analysis of metabolic disorders in animals is controversial. This is usually due to an insufficient understanding or inappropriate interpretation of biochemical results, as well as limitations resulting from differences in methods of samples' collection and the time period between animal death and material collection. Appropriate interpretation of biochemical results has

to be clear and correlated with clinical data and pathological findings. Determination of aqueous humour composition may help in cause of death determination, and appropriate interpretation of causes of pathological lesions found during necropsy. Blood samples collected after death are generally of little diagnostic value, because of physical and chemical changes occurring postmortem. Autolytic changes in aqueous humour occur significantly slower and ocular fluids are relatively easy to collect even few hours after death (Coe 1969; Jaffe 1962; Sturner 1963). Most studies in the field of forensic medicine referred to analysis of potassium level in vitreous humor. It can be helpful in estimation of the time of death (Adelson *et al.* 1963, Adjutantis and Coutselinas 1972; Coe 1969; Hansson *et al.* 1963; Sturner, Gantner 1964). Veterinary studies published during last ten years focused mainly upon correlation between composition of ocular fluids and serum biochemistry results, as well as postmortem changes. They have been conducted on many species including cattle, horses, swine and dogs (Crowell and Duncan 1974; Lane and Lincoln 1985; McLaughlin and McLaughlin 1988). The published data concerned the concentration of particular compounds of eye fluid and their changes in swine in comparison with other species (Drolet *et al.* 1990). Other studies have reported changes in aqueous humour occurring after sample collections until 24 hours after death and they have assessed diagnostic value such analyses. Thus, earlier studies concerned humans, experimental animals (for example monkeys and rabbits) as well as cattle and other species (Crowell and Duncan 1974; Hanna *et al.* 1990; Appleby *et al.* 1990; Aubin *et al.* 2001). Similar analyses may significantly improve postmortem diagnostics of wild animals. Frequent problem in protection of threatened species is the choice of appropriate habitat to meet all environmental conditions of a given population, including its nutritional requirements. It is crucial for homeostasis of threatened species. Its disturbance causes alterations of metabolism including changes in body fluids like blood and aqueous humour. Analysis of eye fluid can be helpful in estimation of the time of wild animal death and can be an useful tool for identification of disorder which animal had suffered before death. Thus, it can help in cause of death determination. However, physiological composition of aqueous humour in particular wild animal species have to be determined before this method will be included in routine diagnostic. Thus, the aim of this study was analysis of concentration of selected chemical elements in aqueous humour in European bison.

Material and methods

Twelve males of lowland European bison living in Białowieża Forest were included in this study. They were obtained through annual culling during autumn-winter from 2013 to 2014. Immediately after culling, all animals were sectioned. Only individuals without any pathological lesions in eye bulb and other changes indirectly influenced eye condition, were included in this study. Then, they were divided into

two groups. The first group consisted of young animals from 3 months to 4 years old. Mature individuals 4–16 years old composed the second group. Samples of aqueous humour were collected during first 4 hours after death by introducing a 19G needle thorough the cornea into the anterior chamber and aspirating fluid into 5 ml syringe. The care was taken to avoid the aspiration of damaged eye structures (for example iris). Then, the material was transported to the laboratory. During 48 hours after collection, all samples were analyzed with colorimetric method using Mindray BS-130 biochemical analyzer. The following parameters were determined: total calcium, ionized calcium, phosphorus, magnesium, sodium and potassium. For each group the mean values of all parameters were calculated. Moreover calcium/phosphorus ratio and sodium/potassium ratio were evaluated.

Results and Discussion

More than half of examined animals had pathological changes in lungs, including pneumonia, pulmonary parasitosis, pulmonary emphysema and bronchopneumonia affecting cranial lobes. In one male 4 months old, the nephritis was diagnosed. In all animals belonging to both age groups, parasites in lung and gastrointestinal tract were found at necropsy. Moreover, necrotising posthitis, focal hepatitis and fasciolosis were diagnosed in the oldest individual. In all animals nor their health history or gross pathology findings did not reveal any signs of metabolic disorders. Thus, we considered all individuals included in this study as representative group regarding the concentration of examined chemical elements in aqueous humour. We assumed the level of all selected molecules as physiological. Therefore obtained data may serve as reference values of various chemical compounds of aqueous humour in European bison. It is relatively easy to collect and analyze of aqueous humour, but correlation between the concentrations of substances in the aqueous humour and in the serum is generally poor, especially considering the evaluation of samples collected on post mortem examination (Edwards *et al*. 2009). Thus, we focused only on analysis of selected chemical compounds in aqueous humour.

Aqueous humour is produced by the ciliary epithelium that lines the ciliary processes located within posterior chamber. It flows from the posterior chamber through the pupil into the anterior chamber. Excess of aqueous humour is drained by the Schlemm's canal into ciliary and conjunctival veins. Aqueous humour supplies nutrients to the lens and the cornea and discharges metabolic waste products. It is formed as a result of diffusion, ultrafiltration and active secretion (Gabelt, Kaufman 2003). Two major factors influence the composition of aqueous humour: diffusion thorough endothelial cells, and the active transport of substances by the ciliary epithelium. Thus, the composition of aqueous humour depends on mechanism involving transudation of serum to the ocular fluid (Cunha-Vaz 2004). Because domestic cattle is the most closely related species to European bison in terms of their

Table 1. Mean values of examined parameters in aqueous humour in both age groups of European bison males.

Groups / Parameter		Group I	Group II
Ionised calcium	[mmol/l]	1,17	1,14
Total calcium	[mg/dl]	5,6	5,28
Ca/P ratio		2,04	1,20
Phosphorus	[mg/dl]	2,93	4,38
Magnesium	[mg/dl]	1,49	1,54
Sodium	[mmol/l]	135,5	142
Potassium	[mmol/l]	5,4	6,1
Na/K ratio		25,10	23,29

metabolism, we decided to analyze in E. bison eye bulbs only the concentration of chemical compounds of the greatest diagnostic significance in ruminants. Thus, the following chemical elements were selected: calcium, phosphorus, magnesium, sodium and potassium (Tabl. 1). The concentration of total calcium and ionized calcium as well as calcium/phosphorus and sodium/potassium ratios were higher in group of immature males comparing to fully mature individuals. For example mature male E. bison had twice higher phosphorus level than young animals. Similarly, concentration of magnesium, sodium and potassium were lower in animals from the first group.

Clinical and biochemical findings may significantly contribute to the cause of death determination. In case of wild animals including European bison, sudden death is often the only clinical sign of illness, and detailed gross examination is required to determine its cause. However, in field conditions, necropsy of fresh carcasses of wild animals is possible very rarely. Blood samples not collected immediately after death are of little diagnostic value, because of haemolysis, breakdown products and postmortem bacterial invasion. The eye is relatively anatomically isolated and protected after death, and ocular fluid deteriorates more slowly than other body fluids (Bito and Salvador 1970). Samples of aqueous humour are relatively easy to collect and can be of value in investigation up to 48 hours after death (James *et al*. 1997). Thus, it seems that biochemical analysis of aqueous humour can be very useful for diagnostic purposes, for example to aid postmortem identification of hypomagnesaemia, that may lead to death. Such studies have been successfully conducted on domestic cattle (Lincoln and Lane 1985). Data on ocular fluids are available for a range of species, including sheep, horses, llamas and alpacas and even manatees (Aubin *et al*. 2001, McCoy *et al*. 2001a; Varela and Bossart 2005). The first report on ocular fluid analysis come from the early 1930s (Salit 1934). In subsequent years, such studies have been continued. They have focused especially on practical

utility of this method and determination of technical conditions of such analysis. The following variables have been studied: the time from death to sample collection and environmental conditions at which carcasses were exposed before ocular fluid collection (McLaughlin and McLaughlin 1987; 1988). More and more attention has been paid to metabolites accumulated in aqueous humour, including urea and creatynine (Lane and Lincoln 1985). Comparison of urea, creatynine and some electrolytes levels in ocular fluids collected postmortem with their antemortem serum levels were also performed (Wilkie and Bellamy 1982). In another study the comparison of beta-hydroxybutyrate concentration in aqueous humour and cerebrospinal fluid in pregnant sheep was done (Scott et al. 1995). Moreover, some other papers were aimed to evaluate whether postmortem levels of urea and chemical elements in aqueous humour can be reliable indicators of antemortem uraemia (Palmer *et al*. 1985; Drolet *et al*. 1990; Wittwer *et al*. 1992). It has been found that apart from compounds physiologically presented in the eye bulb, concentration of toxic substances can also be measured in ocular fluid. It is of particular importance in *postmortem* toxicological diagnostics (Boermans *et al*. 1988; Boermans 1990).

Magnesium and calcium are of the greatest importance in diagnostics of metabolic disorders, especially in ruminants. Because magnesium plays especially important role in biochemical processes in ruminants (Foster *et al*. 2007) many studies have been focused on its distribution and metabolism in bovine ocular fluid under physiological conditions and in hypomagnesaemic animals (McCoy and Kennedy 1994; McCoy *et al*. 2001b; 2001c; McCoy 2004). Metabolism of magnesium, calcium, sodium and potassium in European bison and domestic ruminants are comparable. These chemical elements are considered crucial for metabolic pathways. European bison is a wild animal living in its natural environment. However, increasingly frequent attempts to reintroduce European bison in various regions of the word ended up with various results. Cases of deaths have been even noted. They can be explained by the fact that flora of a given region which does not constitute the natural habitat of European bison can be deficient in chemical elements like magnesium and calcium, crucial for its metabolism. In case of insufficient nutrient intake, results of necropsy of animals found dead are often inconclusive. Moreover, analysis of the composition of natural feedstuff also seems to be incomplete and unreliable. Thus, the best approach in this case is analysis of concentration of specific compounds of aqueous humour within 48 hours after wild animal death. In the current study, only chemical elements causing severe metabolic disorders due to their insufficiency were considered. Analysis was conducted on representative group of male E. bison with various health problems, but without any clinical signs either insufficiency or excess of above mentioned chemical elements. Taking into account, that metabolism of many chemical elements in females is usually influenced by pregnancy and lactation, only males were included in this study. The concentration of magnesium, sodium and potassium was lower in young individuals

comparing to adults. This physiological phenomenon is observed in ruminants, as intense metabolic processes of above mentioned chemical elements develop in fully mature individuals. In contrast, young individuals had lower calcium and phosphorus levels. This observation can be explained by high rate of their metabolism due to intense skeletal development. This indicates, that an appropriate interpretation of analysis of magnesium, calcium and sodium levels in European bison is very complex issue.

Conclusions

Analysis of aqueous humour composition is potentially useful test in postmortem diagnostic in European bison and may provide many valuable information in support of diagnosis. Ocular fluids are among the most accessible samples that may be readily collected after death. It is especially important in wild animals including European bison, as there is often impossible to observe any clinical signs of illness and only carcasses are found. However, the results of analysis of aqueous humour must not be considered in isolation from results of other diagnostic procedures, and any presumptive diagnosis must take account of all other known factors surrounding the death of animal. However, to make this method useful, first the reference values of chemical compounds of aqueous humour in European bison should be established. Thus, further study on larger group of animals, and analysis of more compounds of aqueous humour are necessary.

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Ocena pośmiertna analizy składu chemicznego płynu cieczy wodnistej gałki ocznej u żubra – wyniki wstępne

Streszczenie: Celem pracy było przeprowadzenie oceny przydatności diagnostycznej analizy cieczy wodnistej oka po śmierci zwierzęcia. Próbki płynu zebrano, w ciągu 24 godzin po śmierci, od 12 samców żubrów zamieszkujących tereny Puszczy Białowieskiej. Badane osobniki poddano szczegółowej sekcji zwłok. Zwierzęta podzielono na dwie grupy: pierwsza licząca sześć prób to osobniki młode od 3 miesięcy do 4 lat, druga to zwierzęta dorosłe w wieku od 4 do 16 lat. W ciągu 48 godzin od pobrania wykonano pomiary, metodą kolorymetryczną. Dokonano badań stężenia wapnia całkowitego i zjonizowanego, fosforanu, magnezu, sodu i potasu. Stężenia wapnia całkowitego i zjonizowanego oraz stosunek Ca/P i Na/K w grupie niedojrzałych samców były wyższe niż w grupie samców dorosłych. Natomiast stężenie fosforu było odwrotnie proporcjonalnie i w grupie drugiej dorosłych żubrów i wykazało wartości dwukrotnie wyższe niż w grupie żubrów młodych. Stężenia magnezu, sodu i potasu były niższe w grupie I niż w grupie II. Oznaczanie biochemicznych nieprawidłowości składu cieczy wodnistej oka może być przydatną metodą diagnostyczną do ustalenia przyczyny śmierci lub racjonalnej oceny znaczenia wielu zmian chorobowych stwierdzonych podczas sekcji zwłok. Jednak aby powyższe badania były miarodajne należy ustalić prawidłowe fizjologiczne wartości poszczególnych składników płynów ocznych u żubra.