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Article 16

Review of a Practical Electrometric method for determination of Blood and Tissue Cholinesterase activities in Animals

F. K. Mohammad

Department of Physiology, Biochemistry and Pharmacology,
College of Veterinary Medicine, University of Mosul,
P. O. BOX 11136, Mosul, Iraq
(E-mail: fouadmohammad@yahoo.com)

SUMMARY

Measurement of cholinesterase activity is of diagnostic value in cases of poisoning with organophosphate and carbamate insecticides. The enzyme is inhibited to various extents with concomitant appearance of signs of cholinergic hyperstimulation. The present report introduces and reviews a practical and simple electrometric technique to measure blood (plasma, erythrocyte and whole blood) or tissue (brain, liver and muscle) cholinesterase activities in animals as well as to measure blood cholinesterase activities in man. Typically, the procedure involves the addition of 0.2 ml of blood sample or tissue homogenate to 3 ml of distilled water followed by 3 ml of barbital-phosphate buffer solution (pH 8.1). The pH (pH1) of the mixture is measured, and then 0.1 ml of 7.1% of acetylcholine iodide or 7.5% acetylthiocholine iodide, as a substrate, is added. The reaction mixture is incubated at 37° C for 20-40 minutes according to the animal species. The pH (pH2) of the reaction mixture is measured after the end of the incubation period. The unit of enzyme activity is expressed as Δ pH / incubation time= pH1- pH2 - (Δ pH of the blank). The blank is without the enzyme source. Literature are cited regarding the expected normal cholinesterase activities in man and several animal species including mice, rats, sheep, goats, cattle, chickens, fish and wild birds. The method was found to be efficient, simple, accurate and reproducible for possible monitoring of exposure of man or animals to organophosphate or carbamate insecticides.

KEY WORDS

Carbamate, Cholinesterase, Electrometric method, Insecticide, Organophosphate

INTRODUCTION

Organophosphates and carbamates are widely used insecticides in veterinary medicine, public health and in agriculture (1-3). As a result man and animals are at risk of exposure to these insecticides.

The single most important toxic action of organophosphate and carbamate insecticides is inhibition of acetylcholinesterase activity leading to accumulation of acetylcholine at the nerve endings which in turn produces signs of poisoning characterized by nicotinic, muscarinic and central nervous system effects (1,4,5).

Measurement of blood (plasma or erythrocyte) and tissue cholinesterase activities is a useful tool for monitoring exposure to organophosphate and carbamate insecticides and diagnosing their poisoning (3,6-8). Usually a 20-30% decrease in serum cholinesterase activity suggests exposure to anticholinesterases (9). More than 50% inhibition of cholinesterase activity supports the diagnosis of poisoning and indicates a hazardous condition (6-9). The aim of the present review was to introduce a modified electrometric method advocated for measuring blood or tissue cholinesterase activities in various animal species and to present normal enzyme activity as reported in the literature by our research group.

METHODS FOR MEASURING CHOLINESTERASE ACTIVITY

Various colorimetric and electrometric (potentiometric) methods are available for the determination of cholinesterase activity (6,8,10-14). One of the principle methods for measuring blood cholinesterase activity is the electrometric method which is based on the hydrolysis of acetylcholine and production of acetic acid which in turn decreases the pH of the reaction mixture (6,10,12). The original electrometric method of Michel (15) is commonly used in man (6). However, the method is not efficiently applicable to measure cholinesterase activities of different animal species (6,10,11). This is because of the inherent variations in blood or tissue cholinesterase activities between different animal species (6,11,16-18) and the special need for different buffer compositions, reaction temperatures, incubation times and sample volumes (10,19-21). In addition, the original electrometric method cannot be recommended for detection of cholinesterase inhibition induced by carbamates (10,22,23). Carbamylated cholinesterase is unstable in the reaction mixture of the electrometric method of Michel because of considerable sample dilution and long incubation time (60 min) (10,22,24).

ELECTROMETRIC CHOLINESTERASE DETERMINATION

Various modifications of the electrometric method are available for measuring blood cholinesterase activity in animals (6,10,11,13,14,20,25). These modifications include increasing sample volume, increasing or decreasing incubation time, increasing incubation temperature or using buffers of different compositions (6,10,11). One simple modification of the electrometric method is that of Mohammad et al. (21) which was introduced for rapid measurement of erythrocyte and plasma cholinesterase activities in sheep. The method was then applied successfully on several animal species such as mice (26,27), rats (28-30), goats (31,32), chickens (33-35), wild birds (36) as well as man (27,37,38). More recently, normal reference values for plasma, erythrocyte or whole blood cholinesterase activities as determined by the described electrometric method were reported in man (27,38) as well as in sheep, goats and cattle (39-41).

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Table 1 shows the reported normal blood (plasma, erythrocyte or whole blood) and tissue cholinesterase values in man and different animal species as measured by the presently described method. These cholinesterase values could be starting reference points for future studies applying the presently described electrometric method in biomonitoring of exposure of man and animals to anticholinesterase insecticides. The method is characterized by its simplicity, reproducibility, accuracy and one-step short incubation time (20-40 minutes) depending on the animal species (Table 1). The coefficient of variation of the method is usually low (<10%) (21,27,32). It can be applied on several samples within a relatively short period of time in comparison with the original Michel method. Further, the present method, in contrast to the original electrometric method of Michel (15), can detect cholinesterase inhibition induced by carbamate insecticides such as carbaryl (34,35) and methomyl (28). The method correlates well with the electrometric method of Michel (21,37) and with the colorimetric method of Ellman (34,37,43) in measuring cholinesterase activity. The method also substantially decreases handling of the reaction mixture (e.g., preliminary 10 min incubation time) which is found in other electrometric methods (6,10). In contrast to other electrometric methods, only one type of buffer (barbital-phosphate) solution is used in the present method for the biological samples (21).

PROCEDURE FOR ELECTROMETRIC MEASUREMENT OF BLOOD CHOLINESTERASE ACTIVITY

Venous blood samples are collected using heparinized test tubes (44). Plasma is separated from erythrocytes by centrifugation at 3000 rpm for 15 minutes. Figure 1 outlines the steps for measuring plasma, erythrocyte or whole blood cholinesterase activities. The reaction mixture in a 10-ml beaker contains 3 ml distilled water, 0.2 ml plasma, erythrocytes or whole blood and 3 ml of barbital-phosphate buffer solution (pH 8.1) (21). The pH of the mixture (pH1) is measured with a glass electrode using a pH meter, then 0.10 ml of 7.1% aqueous solution of acetylcholine iodide or 7.5% acetylthiocholine iodide is added to the mixture which is incubated at 37 ºC for 30 minutes. The incubation period in man and cattle is 20 minutes, 30 minutes in sheep, rodents and avian species and 40 minutes in goats (Table 1). At the end of the incubation period, the pH of the reaction mixture (pH2) is measured. The enzyme activity is calculated as follows:

Cholinesterase activity (Δ pH/incubation time) = (pH1 – pH2) - Δ pH of blank

The blank is without the blood sample. The unit of cholinesterase activity is expressed as Δ pH/incubation time, e.g. Δ pH/30 minutes. The barbital-phosphate buffer solution (pH 8.1) consists of 1.237 g sodium barbital, 0.63 g potassium dihydrogen phosphate and 35.07 g sodium chloride dissolved in one liter of distilled water (21). The pH of the buffer is adjusted to 8.1 with 0.1N hydrochloric acid. This buffer solution is suitable for both blood and tissue samples (21,26). For comparison purpose it is possible to use a single incubation period of 30 minutes for sheep, goats and cattle (41).

PROCEDURE FOR MEASURING TISSUE CHOLINESTERASE ACTIVITY

Samples (0.5-1 g) of brain, liver or muscle are homogenized in the barbital-phosphate buffer solution (pH 8.1) at 100 mg wet tissue weight/3 ml with a teflon homogenizer using 25% of the maximum velocity of the electric homogenizer (26,29,34). Glass homogenizer can also be used for manual homogenization. Homogenization is performed on an ice bath, and all tissue homogenates are kept on ice before cholinesterase determination. For tissue cholinesterase activity, 0.2 ml of the tissue homogenate is used instead of the blood aliquot in the reaction mixture described above. The rest of the procedure is the same as in the case of the blood (Figure 2).

MONITORING EXPOSURE TO ORGANOPHOSPHATE OR CARBAMATE INSECTICIDES

The described electrometric method was reported to be efficient in detecting in vitro or in vivo cholinesterase inhibition in the blood or tissues of man and different animal species (21,27,28,29,34,35,38,45). Veterinarians and agriculture workers exposed to insecticides during their routine work had relatively low plasma and erythrocyte cholinesterase activities as detected by the present electrometric method (27,45). The method was also utilized to detect the extent of blood or tissue cholinesterase inhibition in animals intoxicated experimentally with organophosphate insecticides such as dichlorvos (27,29,34,35), diazinon (31,43,46) and malathion (33,45) as well as with carbamate insecticides such as carbaryl (27,34,35) and methomyl (28). The method was also applied to detect plasma or tissue cholinesterase inhibition in wild birds (rock dove, quail, sand grouse and starling) (43) and chickens (33-35) intoxicated with organophosphates and carbamates and in fish (common carp) intoxicated with cypermethrin (42). These results in birds and fish suggest the possibility of applying the present electrometric method for biomonitoring of environmental contamination with anticholinesterase compounds by assessing plasma or tissue cholinesterase inhibition in wild birds which are frequently exposed to insecticides as a result of human activities (1,16,47). Furthermore, cholinesterase measurement in domestic animals has been advocated as a potential biomonitoring tool for organophosphate exposure (14,48,49).

OTHER APPLICATIONS

Another important application of the present cholinesterase method could be evaluation of the enzyme activity in the amniotic fluids. This would be highly economic screening test as cholinesterase determination in amniotic fluids can provide an index of suspected fetal neural tube defects.

CONCLUSION

The described electrometric method could be an added simple and practical method for measuring blood or tissue cholinesterase activity in man and animals exposed to organophosphate and carbamate insecticides. The method has the potential for application in biomonitoring of environmental exposure of wild birds or domestic animals to anticholinesterase pesticides.

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Table 1

Cholinesterase activity determined by the described electrometric method in man and animals

Species	Sex	Cholinesterase	Incubation time (minutes)	Activity (ΔpH)	Reference
Man	male	plasma	20	1.05	27,37
		erythrocyte	20	1.18	
		whole blood	20	1.25	
	female	plasma	20	0.91	
		erythrocyte	20	1.19	
		whole blood	20	1.23	
Mice	female	whole blood	30	1.10	26
		brain	30	0.39	
		liver	30	0.39	
Rats	male	plasma	30	0.29	29
		erythrocytes	30	0.29	
		brain	30	0.19	
	female	plasma	30	0.26	28
		erythrocytes	30	0.30	
	female	plasma	30	0.40	30
		erythrocytes	30	0.28	
		brain	30	0.26	
Sheep	mixed	plasma	30	0.09	21

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		erythrocytes	30	0.70	
	male	plasma	30	0.21	39
		erythrocytes	30	0.63	
	female	plasma	30	0.19	
		erythrocyte	30	0.62	
Goats	mixed	plasma	40	0.19	31,32
		erythrocytes	40	0.42	
	male	plasma	40	0.22	
		erythrocytes	40	0.43	
	female	plasma	40	0.19	
		erythrocytes	40	0.36	
	male	plasma	40	0.22	39
		erythrocytes	40	0.54	
	female	plasma	40	0.22	
		erythrocytes	40	0.44	
Cattle	male	plasma	20	0.10	39
		erythrocytes	20	0.91	
	female	plasma	20	0.19	
		erythrocytes	20	0.86	
Chickens	mixed-chicks	plasma	30	0.56	34
		brain	30	0.34	

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		liver	30	0.15	
		muscle	30	0.12	
	male				
		plasma	30	0.54	33
	mixed				
		plasma	30	0.47	35
		brain	30	0.25	
		liver	30	0.21	
Rock dove	mixed	plasma	30	1.28	36
		brain	30	0.59	
		liver	30	0.12	
		muscle	30	0.08	
Pin tailed sandgrouse	Mixed	plasma	30	1.81	36
		brain	30	0.37	
		liver	30	0.06	
		muscle	30	0.07	
Quail	Mixed	plasma	30	1.23	36
		brain	30	0.39	
		liver	30	0.19	
		muscle	30	0.06	
Starling	Mixed	plasma	30	1.10	36
		brain	30	0.24	
		liver	30	0.08	

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		muscle	30	0.08	
Fish (*Cyprinus carpio*)	Mixed	Brain	30	0.60	42

Figure 1: Steps for the electrometric determination of blood cholinesterase activity

3 ml distilled water

+

0.2 ml aliquot of plasma, erythrocytes or whole blood

+

3 ml barbital-phosphate buffer (pH 8.1)

⇩

Measure pH (pH1)

⇩

Add 0.1 ml 7.1% acetylcholine iodide

⇩

Incubate at 37 ºC (e.g. 20 min in man, 30 min in birds)

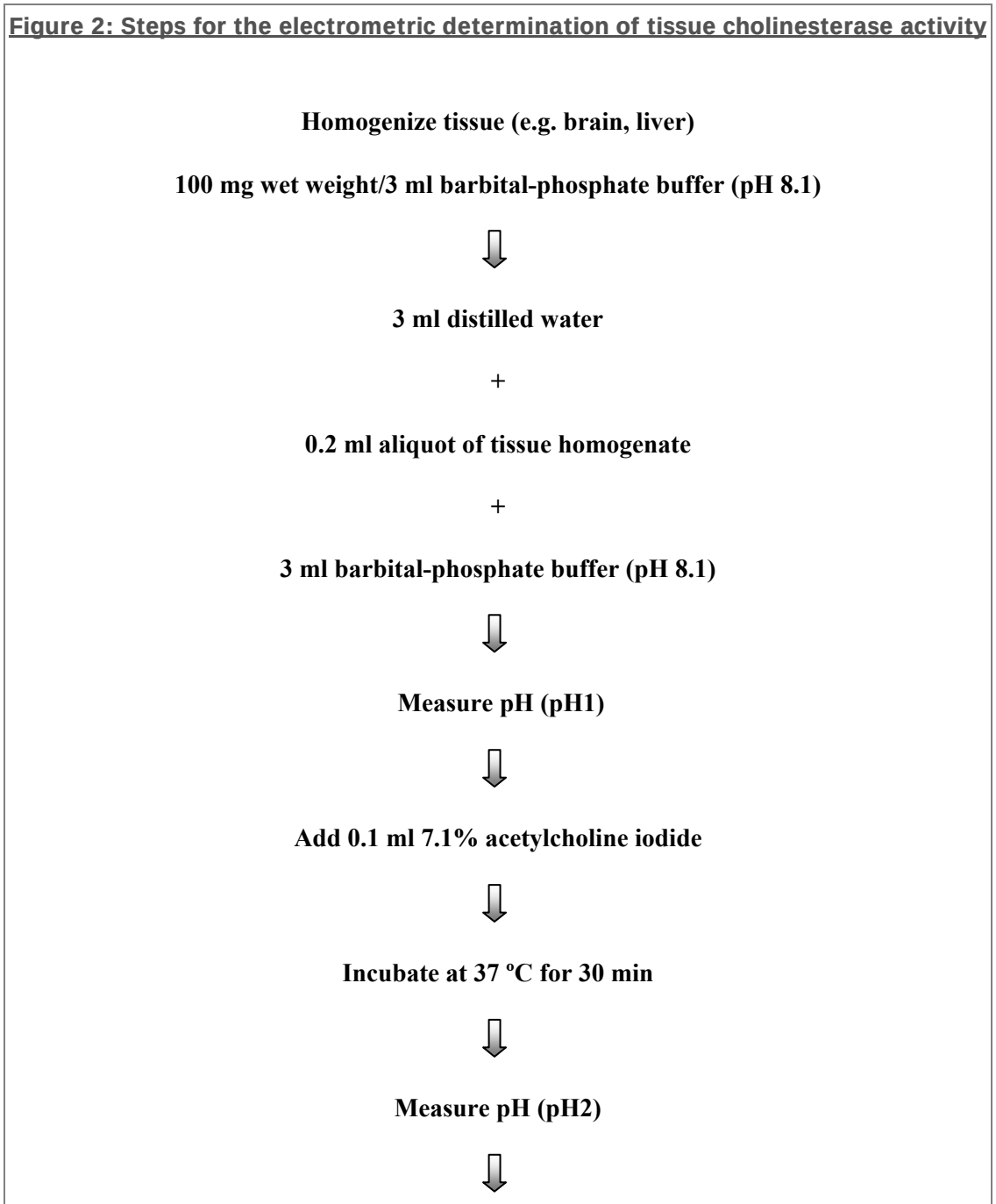
⇩

Measure pH (pH2)

⇩

Cholinesterase activity (Δ pH/incubation time)= pH1-pH2- (Δ pH of blank, without sample)

Figure 2: Steps for the electrometric determination of tissue cholinesterase activity



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Article 17

Management of Toxic Puerperal Metritis in Dairy Cows using Oxytetracycline along with PGF2α Therapy

H. K. Bhattacharyya and M. R. Fazili

Teaching Veterinary Clinical Complex,
Faculty of Veterinary Sciences & Animal Husbandry, Shuhama, Alustang,
Srinagar-190006 (J&K)

ABSTRACT

Successful treatment of toxic puerperal metritis in cattle with intrauterine infusion of oxytetracycline and parenteral administration of PGF2α is reported and discussed.

KEY WORDS

Toxic puerperal metritis (TPP), Oxytetracycline, PGF2α.

INTRODUCTION

Toxic puerperal metritis (TPP) is a reproductive disorder affecting dairy cattle. It results in heavy economic loss due to decreased milk yield, reduced fertility and high treatment cost. Incidence of the disease varies from 2.2 to 37% (Kelton et al., 1998). Various agents have been used to treat this condition from time to time which includes oxytetracycline, penicillin, sulfonamide, β-lactams (Whitacre, 1992; Oslon et al., 1986), ceftiofur (Smith et al., 1998), hepatoglobins, cytokines (Alsemgeest et. al., 1994), prostaglandin F2α (Melendez et al., 2004) etc. The purpose of this communication is to put on record the efficacy of oxytetracycline along with PGF2α in 8 cows suffering from TPM.

CASE HISTORY AND OBSERVATION

A total of 8 cows in 2nd to 5th lactation were presented within 10-14 days post-calving with the history of anorexia, depression, mild dehydration, reduced milk yield and watery foetid vulvar discharge. All the cows were feverish (103-104.50F) and their per rectal examination revealed flaccid, non-retractable uterus that were located in the abdomen along with cervical enlargement. Cervical diameter (>75mm) was assessed following the criteria fixed by Zemjanis (1970). In 4 cows caruncles were not involuted (1-2 cm in diameter) with very big middle uterine artery (m.u.a.) that gave characteristic fremitus like pulsation resembling with the cases of superfoetation. Five cows had suffered from retained foetal membrane that was removed manually by local veterinarian. Two more cows developed milk fever on 2nd day post-calving. Uterine discharge was collected aseptically and subjected to sensitivity testing.

TREATMENT

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Cows were treated with intrauterine infusion of oxytetracycline (Liquid Terramycin* 60 ml daily for 5 days) and PGF2α (Lutalyse** 5 ml i/m) on the first day of oxytetracycline administration. No other treatment was provided.

(* 50 mg/ml, Pfizer Animal Health Division, Mumbai – 400 021, ** 5 mg/ml, Novartis India Limited, Animal Health Sector, Mumbai – 400 020)

RESULTS AND DISCUSSION

The treatment resulted in expelling of discharges amounting to 10 to 15 liters from all the cows within 3 days. Uterine involution had progressed to around 50% on day 3 and 70% on day 5. Caruncles were involuted completely on day 5. Rectal temperature reduced to normal values and m.u.a. returned to non-gravid state. All the animals resumed feeding.

Toxic puerperal metritis is a disease characterized by fever, anorexia, depression, dehydration, reduced milk yield and foetid watery uterine discharge (Oslon et. al., 1986; Smith et. al., 1998). Similar findings were recorded in the present study. However, status of m.u.a. and caruncles in cases of TPM was not mentioned in the earlier studies. In the present study incomplete involution of m.u.a. and caruncles was observed in 50% cases. Therefore, in future TPM may be defined on the basis of all criteria including status of m.u.a., caruncles and dehydration in addition to the other previously fixed criteria.

The disease developed within 10-14 days post-calving i.e. early in the post-partum period. This finding simulates with the earlier finding of Gilbert and Schwark (1992) who reported that the disease occurred within 1st week post-partum. The TPM is observed as acute condition due to involvement of highly pathogenic organism and other predisposing factors like poor resistance of the cow.

In the present study 5 out of 8 cows (62.5%) had suffered from retained foetal membrane and 2 cows (25%) suffered from milk fever. Association of retained foetal membrane and metritis was also reported earlier (Smith et al., 1998). Retained foetal membrane and hypocalcemia may act as a contributing factor for metritis by decreasing required uterine motility which is caused by reduction in plasma calcium level.

It is very difficult to determine the exact cause of TPM because of fastidious nature of the organisms and due to involvement of both pathogenic and non pathogenic organisms. However, most researchers reported involvement of both gram positive and gram negative bacteria and hence systemic and/ or intrauterine infusion of antibiotic is must to kill the involved organisms. In the present study oxytetracycline was used to which all fluid samples showed high sensitivity. Oxytetracycline for the treatment of acute metritis was also advocated earlier by several workers (Gustafson, 1986; Whitacre, 1992).

Normal myometrial activity of the uterus is greater at calving and decreases drastically around 7 to 9 days post-partum (Gajewski et al., 1999). Although inflamed uterus (metritis) produces additional prostaglandin, the uterine musculature does not respond to this endogenous prostaglandin and involution process is delayed (Kindahl et al., 1999). Calcium is a key mediator for muscle contraction (Nelson and Cox, 2000). Cows that develop hypocalcaemia within few days post-partum have prolonged intervals to

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complete uterine and cervical involution than normocalcaemic cows (Kamgarpour et al., 1999). In such cows uterotonic compounds like PGF2α might be less effective in triggering uterine motility and tonacity (Melendez et al., 2004). In the present study exogenous administration of PGF2α by day 10-14 played a vital role in clearing debris from the uterus by increasing myometrial activity and uterine contractility (Patil et al., 1980; Gajewski et al., 1999). From the study it is concluded that intrauterine administration of oxytetracycline along with parenteral PGF2α is highly useful in treating TPM in dairy cows.

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Article 18

Comparative Efficacy of Two Preparations of Albendazole against Natural Gastro Intestinal Strongyle Infestation of Sheep in Kashmir Valley

S. Nasreen, Syed Ghulam Jeelani and Nazir Ahmad Sheikh

Division of Parasitology, Disease Investigation Laboratory,
Sheep Husbandry Department, Kashmir, India

ABSTRACT

A study of the comparative efficacy of two preparations of albendazole against gastrointestinal strongyles in sheep was conducted. The eggs per gram (EPG) values were recorded pre and post treatment and percentile reduction for the two formulations were compared.

KEYWORDS

Albendazole, Micronised Albendazole, Strongyles, Sheep

INTRODUCTION

The practice of sheep grazing in open post-harvest paddy fields and alpine pastures of Kashmir valley results in heavy nematode infestation. Such infestations lead to high morbidity and low productivity in the affected animals. The seasonal migration of sheep also plays an important role in further dissemination of nematode eggs and larvae. Periodic anthelmintic dosing under such conditions becomes a necessity. Different formulations of the same drugs may affect its efficacy and some manufacturers claim better results with their formulation. In the present trial the results of comparative efficacy of albendazole and micronized albendazole against natural gastrointestinal strongyle infestation are reported.

MATERIALS AND METHODS

Thirty sheep of either sex infected naturally with gastrointestinal strongyles were selected from Burzahama and Zakura area of District Srinagar. These animals were randomly divided into three groups A, B, and C. Group "A" animal were administered no anthelmintic and acted as control. Group "B" animals were treated with a regular albendazole suspension and Group "C" animals with micronized albendazole, both administered orally at the same dose rate of 7.5 mg/ kg body weight.

The eggs per gram (EPG) values of faecal samples were recorded in all animals before treatment (day 0) and on the 7th, 14th, & 21st day post-treatment by Stoll's dilution technique (Soulsby 1982). The efficacy of the anthelmintic preparation was calculated by comparing the means values of EPG before treatment with their values at different post treatment intervals. (Taylor et al, 1993)

$$\text{Percent Efficacy} = \frac{\text{Mean EPG pre treatment} - \text{Mean EPG post treatment}}{\text{Mean EPG pre treatment}} \times 100$$

RESULTS AND DISCUSSION

In the untreated control animals (Group A) the strongyle EPG values showed a steady increase in count from day 0 to day 21. In groups "B" and "C" the EPG count values showed reduction which continued from day 7 to 21st post treatment (Table 1)

The percent reduction in EPG in animals treated with commonly available albendazole was 87.16, 92.27 and 97.74 on days 7, 14, and 21 respectively. These values were marginally higher (87.72, 98. 03 and 99.01) in animals treated with micronized albendazole. Similar values were reputed by Godbole et al (1998) Verma et al (1998) and Khillare et al (2002).

The present study indicates that albendazole is effective as an anthelmintic against ovine strongyle infestations and that minor improvement in efficacy is obtained with administration of micronized formulation of the drug.

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Table 1

Percent reduction of fecal EPG of strongyle specie with two preparations of albendazole

Group	No. of Animals	Drug with dose and route	Mean EPG							
			Pre- treatment EPG		Post-treatment EPG					
			0 Day	Reduction %	7th day	Reduction %	14th day	Reduction %	21st day	Reduction %
A	10	Control (untreated)	1147.0	--		--	1250.00	--	1350.00	--
B	10	Albendazole 7.5 mg/ kg Bwt orally	1286.6	--	165.00	87.16	35.00	97.27	29.00	97.74
C	10	Micronised Albendazole 7.5 mg/ kg Bwt orally	1018.0	--	125.00	87.72	20.00	98.03	10.00	99.01

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Article 19

Plasma Glucose and Insulin Profiles in Ketotic Buffaloes

Shabbir A. Teli[1] & S. L. Ali[2]

Department of Veterinary Medicine, College of Veterinary Sciences, Anjora, Durg (CG), India
(Part of MVSc research work, Thesis submitted to IGKVV, Raipur)
[1]Department of Animal Husbandry, Kashmir
[2]Associate Professor, Department of Veterinary Medicine, College of Veterinary Sciences, Anjora, Durg (CG), India

ABSTRACT

A simultaneous decrease in the values of plasma glucose and insulin was observed in 24 ketotic buffaloes. The mean glucose level before treatment was estimated to 37.68 ± 0.84 mg/dl, whereas insulin concentration estimated to a mean of 15.82 ± 0.35 µu/ ml. At clinical recovery elevated values to a mean of 59.00 ± 1.31 mg/dl and 24.37 ± 1.51 µu/ ml respectively were estimated. Thus resulting in a corresponding improvement in the values of both parameters.

KEYWORDS

Ketosis, Glucose, Insulin, Buffaloes

INTRODUCTION

In a heavy milking animal 60% to 80% of the blood glucose is utilized by the mammary glands in the production of milk (Annison and Linzell, 1963). Normally ruminants have low levels of blood glucose and even slight falls are enough to put the animal in a hypoglycemic state. If not relieved the animals metabolism shifts and further blood bio-chemical alterations including elevated ketone bodies, free fatty acids, tri-glycerides and cholesterol with decrease in calcium and insulin are seen (Singh and Kasaralikar, 1990 ; Sakai et al, 1996) and clinically the animal is presented as ketotic.

The present study evaluates plasma glucose and insulin levels before and after successful treatment of ketotic buffaloes.

MATERIALS AND METHODS

The study was conducted on twenty four clinically ketotic buffaloes. The animals were randomly allotted to four treatment groups (Table 1). Paired blood samples were aseptically collected by venipuncture, in vials containing heparin and sodium fluoride respectively and carried to laboratory on ice. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20º C till further testing.

Blood glucose (mg/dl) was estimated by GOD/POD (enzymatic) method (Tietz, 1976) whereas, plasma insulin (µu/ ml) concentration was determined by ELISA as per Sacks (1994).

The biochemical changes were evaluated by analyzing the data using paired "t" test as per standard procedures out lined by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The mean values of plasma glucose and insulin prior to treatment were 37.68 + 0.84 mg/dl and 15.82 + 0.35 µu/ml. A corresponding and significant ($p< 0.01$) increase in the values of both the parameters was observed following the different treatment regimens. The means recorded at clinical recovery were 59.00 + 1.31 mg/dl and 24.37 + 1.51 µu/ml. The intra assay coefficient of variation was estimated to be 8.53.

The present observations corroborate the findings of Hove (1974) who reported a corresponding decrease in plasma glucose and insulin in ketotic cows. Decrease in palsma glucose was earlier reported by Kronfeld (1980), Chugh et al (1992) in cows and Anantwar & Singh (1993), Ambore et al (2001) and Mandali et al (2002) in lactating buffaloes. Hypoinsulinemia remains a constant feature in a ketotic animal (Kolb, 1977). The decrease in the insulin levels could be attributed to the diminished ability of β–cells of endocrine pancreas to synthesize and release insulin (Hove 1978 , Dokovic at al 1998).

During the present trial, administration of glucose in conjugation with insulin/dextran elevated blood glucose levels which might have in turn triggered the synthesis and further release of insulin from the pancreas.

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Table 1

Design for therapeutic evaluation in bubaline ketosis

Group	Therapeutic regimen	Dose and route	Duration
I	Inj. Dextrose [A] 25%	Two pints (540mlx2) i/v	2-3 days
II	Inj. Dextrose [B] 25% + Inj. Insulin [C]	Two pints (540mlx2) i/v 200 iu s/c	1-2 days
III	Inj. Xylitol [C] 25% + Inj. Dextrose [A] 25%	540 ml i/v 540 ml i/v	1-2 days
IV	Liquid Glucose [D] + Sodium bicarbonate	500g Orally 30 g Orally	2-3 Days

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Article 20

An Outbreak of a Haemorrhagic Syndrome in Poultry

K. A. Shah[1] & S. Qureshi[2]

[1]Laboratory Officer (Poultry), Disease Investigation Laboratory,
Institute of Animal Health & Biological Production, Zakura,
Animal Husbandry Department-Kashmir, Srinagar, Kashmir, India
[2]Assistant Professor, Division of Veterinary Microbiology & Immunology,
Faculty of Veterinary Sciences & Animal Husbandry (SKUAST-K),
Shuhama, Alusteng, Srinagar, Kashmir, India

ABSTRACT

An outbreak of a haemorrhagic disease was noticed in 5-week-old chicks at a poultry farm in Srinagar. Escherichia coli and Staphylococcus were isolated from morbid material following culture. Administration of Cephalexin and a haematinic tonic and homeopathic medicines Ficus religiosa Q, Arnica montana 200 in combination with Ascorbic acid therapy controlled the mortality within 6 days limiting it to 15%.

KEYWORDS

Haemorrhage, Escherichia coli, Staphylococcus, Poultry

INTRODUCTION

Poultry birds are prone to blood dyscrasias which are manifested as haemorrhages in muscles, internal organs, skin and sometimes changes in bone marrow. Immunosuppression of young chicken by diseases like infectious bursal disease and coccidiosis as well as other conditions of acute stress, also prolonged use of sulfa drugs, deficiency of Vitamin K may predispose to such syndromes. (Calnek et al., 1991, Gove Hambidge, 2004).

Meager information is available on the incidence of such syndromes in the state of Jammu & Kashmir. The present study elucidates the clinico-pathological studies and management of an outbreak of a haemorrhagic syndrome in poultry birds.

MATERIALS AND METHODS

Heavy mortality was encountered in 5-week-old chicks reared on deep litter system at a commercial poultry farm having flock strength of 2500. Management practices, clinical signs and mortality patterns were noticed.

Tissue pieces from visceral organs were aseptically collected during post-mortem examination and subjected to culture sensitivity testing.

RESULTS AND DISCUSSION

The birds exhibited signs of dullness, drooping, ruffled feathers, closed eyes, incoordination and weight loss which is in accordance with findings of Chowdary et al, (2004). The overall mortality was 15% and consistent lesions noticed at necropsy were bursal atrophy, deep scattered haemorrhages on pale and anaemic muscles of thigh, leg, breast and skin, besides punctate haemorrhages on ochre coloured liver, anterior chamber of eye, intestines and pericardial sac. The femoral bone marrow exhibited paleness. Focal haemorrhages were evident on spleen, kidney and pro-ventricular junction with gizzard.

Escherichia coli and Staphylococcal organisms were isolated from the morbid tissue material collected. Antibiograms of the isolates revealed resistance of the organisms to tetracyclines, amoxycillin, ampicillin and gentamicin whereas sensitivity to norfloxacin, cephalexin, cefadroxil, sparfloxacin and amikacin was observed with maximum zone of inhibition for cephalexin and amikacin and minimum for ciprofloxacin.

The affected flock had been reared on a damp litter with inadequate ventilation besides, an above normal ammonia level. The flock had also a previous history of coccidiosis outbreak. It may be presumed that such stress conditions could have caused a certain degree immunosuppression, predisposing the flock to the haemorrhagic syndrome.

Changing litter and providing adequate ventilation and floor space did revival of managemental practice. Homeopathic medicine Ficus religiosa @ 3 drops/lit of drinking water in morning and Arnica montana 200, @ 10 drops /litre in evening were prescribed for 3 days. This therapy was followed with cephalexin 500mg/lit in combination with 10 ml of haematinic, Haem-up (containing ferric ammonium citrate 160 mg, cynacobalamine 7.5mg, folic acid 0.5mg, cupric sulphate 30mcg/15ml) and 50mg of ascorbic acid/lit of drinking water for 4 days. Mortality was controlled within 6 days limiting it to 15%.

Ficus religiosa mimics the action of coagulating factors whereas Arnica montana increases phagocytosis thus mobilising blood clots and haematomas. In young chicken, biosynthesis of ascorbic acid is limited and its requirement is increased greatly during stressful conditions (Kutulu and Forbes 1994, Ravinder, 2004). Ascorbic acid plays a vital role in functioning of folic acid and cyanocobalamine to maintain normal haemopoiesis in bone marrow and elsewhere in body. Apart from maintaining capillary functions it also increase immunity under stress conditions (Brander et al., 1982, Ravinder, 2004) .In view of these reports and present finding it may be safely inferred that the cumulative action of homeopathic medicines and haematinics and ascorbic acid therapy might have brought the outbreak under control.

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