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Pharmacodynamic Interaction of Doramectin with Acepromazine in Mice

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Abstract Doramectin, a novel avermectin, is widely used as a potent endectocide. There are evidences which show that doramectin has effects on the central nervous system of the animals, since it affects GABA sensitive neurons. The present study was conducted to examine the pharmacodynamic interactions that may result from concurrent administration of doramectin and acepromazine, a tranquillizer. In this experimental study, the effect of doramectin on the activity of acepromazine was assessed by employing Spontaneous locomotor activity test, Forced locomotor activity test, Traction wire test, Conditioned response avoidance test and aggregation toxicity. In spontaneous locomotor activity test it was found that doramectin potentiated the activity of acepromozine but it was not significant. In forced locomotor activity test and traction wire test it revealed that doramectin attenuated the activity of acepromazine but again not significant. The result of conditioned response avoidance test and aggregation toxicity test showed that doramectin did not alter the activity of acepromazine. Thus, the results obtained in the present study suggest that doramectin doesn't affect the pharmacologic activity of acepramozine. This could be attributed to the difference in the mechanism of action of the two drugs in the central nervous system.
Keywords *Acepromazine, Pharmacodynamics, Doramectin, GABA, Central Nervous System*

1. Introduction

Doramectin is a new substance of the chemical class avermectins produced by submerged aerobic fermentation of the soil actinomycete Streptomyces avermitilis. It is used as a potent novel veterinary endectocide i.e. combined killing of both endo-parasites and ecto- parasites. Evidences show that doramectin has effects on the central nervous system (CNS), since like ivermectin and other drugs in this class it affects GABA (GABAA)-sensitive neurons, which can lead to neurotoxicity, as tremors,

ataxia and gait abnormalities [1, 2]. The neurotransmitter GABA is an important inhibitory neurotransmitter in mammalian brain [3]. It was suggested that doramectin has the pharmacological profile of an anxiolytic/anticonvulsant drug with GABAergic properties [4].

Acepromazine is one of the most commonly used tranquilizers in veterinary medicine [5, 6]. It is a phenothiazine compound. Its mode of action is only partially understood but it involves blockage of dopamine nerve receptors in the brain. It causes tranquilization and also has an anti-emetic effect. There are reports of preliminary investigations on pharmacodynamics interactions of ivermectin with the CNS acting drugs. But there are very few studies on the pharmacodynamics interactions of doramectin with acepromazine.

The present study was undertaken to understand the possible pharmacodynamics interaction of doramectin with acepromazine, a tranquillizer, in mice.

2. Materials and Methods

2.1. Animals

Male albino mice, weighing 20-25 grams were procured from Central Animal Facility of NIPER, Mohali. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 h light-dark cycle in the departmental animal facility. Animals were given an acclimatization period of seven days before commencing first experiment. Food and water were provided ad libitum. Food was withdrawn 12 h before and during the experiment. Experiments were performed during 8 AM to 12 PM. All experimental protocols were approved by the institutional animal ethics committee (IAEC) and "Guide for the care and use of laboratory animals" approved by the National Research Council (ILAR) in USA will have been observed.

2.2. Drugs

The following drugs were used: doramectin (Dectomax®, Pfizer Inc., USA), Acepromazine (Ilium acepril, Troy labs, Australia and Amphetamine (Amphetamine, Merck, USA). Doramectin was injected subcutaneously at two doses 200 μg/kg (normal dose) and 600 μg/kg (three times the normal dose); Acepromazine (@ 2 mg/kg) and amphetamine (15 mg/kg) were injected intraperitoneally. The observations were recorded on the 5th and 6th day since doramectin has a half-life of 1 week.

In this study, twenty four animals were divided into four groups with 6 animals in each group as follows:

Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Acepromazine (2 mg/kg),
Test 1 -Doramectin 200 μg/kg + Acepromazine (2 mg/kg),
Test 2 -Doramectin 600 μg/kg + Acepromazine (2 mg/kg).

2.3. Spontaneous Locomotor Activity (SLA) Test

The mouse is put in the dark chamber of the equipment and turned on. Being a nocturnal, the mouse starts moving in the chamber instinctively. Movement of the animal cuts through a beam of light and the photocell gets activated and this count was recorded digitally. The standard acepromazine was administered 20 min prior to performing the trial. The SLA score of animals was recorded for 10 minutes [7].

2.4. Forced Locomotor Activity Test

Rota rod test was used to assess the forced locomotor activity (FLA) in the mice and were observed for ability to maintain equilibrium and muscle control on the rotating rod. The rate of revolution of the rod was adjusted at three different speeds, viz., low (5 rpm), medium (15 rpm) and high (25 rpm). The mice were initially trained on the rota rod at low speed for 5 min on 2-3 occasions. For further test, only those mice, which completed three successful trials per day for 2 days without falling off the rod, were selected. The standard acepromazine was administered 20 min before the trial. The animals were observed for fall offs and free rides and respective time taken was noted [8].

2.5. Traction Wire Test

Traction wire is a forced activity and has been used to access the ability to maintain equilibrium and muscle control on a wire tied between two poles. In this the mice were placed with their forelimbs on the wire and trained to use their hind limbs to maintain their equilibrium. Normal mice were able to maintain their equilibrium by using both fore and hind limbs and forming a ball like appearance. The mice were trained to maintain their stability on the traction wire previously. The time taken to fall off was taken as end point. The animals were administered standard acepromazine 20 min before the actual trial.

2.6. Conditioned Response Avoidance Test

In this protocol, the mice were subjected to a series of conditions such as light, followed by buzzer and finally leading to shock. Initial training for 3 days was given to the mice and only those mice that responded to the conditioned stimuli by climbing on the pole to avoid the shock were chosen. The standard tranquillizer was administered 20 minutes prior to the trial and the mice were tested for conditioned response by applying buzzer and shock. Failure of the mice to climb the pole after putting on the light and sounding the buzzer, followed by shock was considered as the end point [9].

2.7. Aggregation Toxicity

This test tends to create a fighting behaviour induced by a CNS stimulant in the mice when kept in more numbers in a cage. The response is recorded was observed as piloerection, increased motor activity, fighting behavior and convulsions. The standard protective drug, i.e. tranquillizer, was injected in experimental groups and after 20 min the amphetamine was administered and the animals were observed for aggregation toxicity for 30 minutes [7].

2.8. Statistics Analysis

Results are presented as mean ± standard error of mean (SEM). The level of significance was taken as: $p<0.05$ and $p<0.01$ as significant; $p<0.001$ highly significant.

3. Results

In this experimental study, the effect of doramectine on the activity of acepromazine was assessed by employing spontaneous locomotor activity test, forced locomotor activity test, traction wire test, conditioned response avoidance test and aggregation toxicity test.

3.1. Spontaneous Locomotor Activity Test

Spontaneous locomotor activity (SLA) is measured using photoactometer, which records the active exploratory movements of the animals, and is the commonest method used to screen sedatives or CNS drugs. The photoactometer readings recorded in different groups are as shown in the table 1.

***Table 1**: Effect of acepromazine on SLA of mice pretreated with doramectin*

Groups (n=6)	Photoactometer readings in minutes									
	1	2	3	4	5	6	7	8	9	10
Control 1	75.17 ±4.51	53.00 ±4.73	42.83 ±6.83	41.00 ±2.62	37.17 ±3.13	37.17 ±4.90	33.83 ±4.41	28.00 ±1.73	30.33 ±6.28	30.33 ±3.69
Acepromazine treatment (2 mg/kg b.wt.)										
Control 2	33.50 ±8.36	22.83 ±13.14	12.83 ±5.71	12.67 ±6.44	8.50 ±3.62	14.83 ±5.19	15.67 ±5.32	10.17 ±2.79	13.50 ±4.04	9.33 ±2.73
Test 1	23.67 ±6.13	26.33 ±4.17	12.83 ±4.50	10.17 ±5.78	8.83 ±2.63	10.00 ±1.15	9.83 ±1.28	7.00 ±0.68	6.33 ±0.76	7.67 ±1.33
Test 2	30.00 ±5.18	25.00 ±3.35	17.17 ±5.60	13.33 ±6.51	12.50 ±4.68	12.33 ±3.09	6.00 ±0.86	6.50 ±0.67	9.00 ±4.03	8.33 ±2.25

* p<0.05; ** p<0.01, indicates significant difference when compared with their respective control values

3.2. Forced Locomotor Activity Test

Rota rod test was used to assess the forced locomotor activity (FLA) in the mice and were observed for ability to maintain equilibrium and muscular coordination on the rotating rod. The observation for fall off was made at 3 different speeds and is presented in the Table 2.

***Table 2:** Effect of acepromazine on Rota rod treadmill induced FLA of mice pretreated with doramectin. (time taken in seconds)*

Groups (n=6)	Low (5 rpm)	Medium (15 rpm)	High (25 rpm)
Control 1	No fall	No fall	No fall
Acepromazine treated (2 mg/kg b.wt. ip)			
Control 2	No fall	182.33 ± 26.23	49.83 ± 7.40
Test 1	No fall	181.50 ± 24.20	58.17 ± 7.19
Test 2	No fall	172.83 ± 22.03	59.83 ± 3.83

* p<0.05, indicates significant difference when compared with their respective control values

It was found that in the untreated control group did not show any fall or free ride at all the speeds. On treatment with acepromazine, all the groups did not show any fall off at low speed of 5 rpm. However at medium (15 rpm) and high (25 rpm) speed all the mice showed fall off at increasing time interval.

3.3 Traction Wire Test

Traction wire was used as another method to assess the forced locomotor activity. The inability to maintain equilibrium and time taken to fall off was taken as end point. In this test, the mice were able maintain their equilibrium in control group by holding the traction wire with all the four limbs and forming a ball like structure. However on acepromazine treatment, all the animals showed fall off at a short interval. The result is presented in the Table 3.

Table 3: *Effect of acepromazine on traction wire FLA of mice treated with doramectin*

Groups (n=6)	Fall off time (sec.)
Control 1	No fall
Acepromazine treated	
Control 2	34.00 ± 4.32
Test 1	35.17 ± 3.12
Test 2	47.50 ± 6.19*

* p<0.05, indicates significant difference when compared with the respective control values

3.4. Conditioned Response Avoidance Test

In this test, the rats were subjected to a series of conditions such as light, followed by buzzer and finally electric shock. It was found that mice from control group responded to conditioned stimuli by climbing the pole. Acepromazine at anxiolytic dose was able to block the conditioned response in all the groups. The result is presented in the table 4.

Table 4: *Effect of acepromazine on conditioned response test (pole climbing) in rats treated with doramectin. (Value in parenthesis show number of rats responding to conditioned stimulus and climbing the pole)*

Groups (n=6)	***Response to…***		
	Light	**Buzzer**	**Shock**
Control 1	Present	Present	Present
Acepromazine (1 mg/kg b.wt.)			
Control 2	Absent	Absent	Absent (1)
Test 1	Absent	Absent	Absent (1)
Test 2	Absent	Absent	Absent

3.5. Aggregation Toxicity

In this test Amphetamine, a CNS stimulant, was used to induce a fighting behaviour in the mice when kept in more numbers in same cage. Acepromazine, standard protective drug, was able to protect the mice against aggregation toxicity in all the groups. There were no convulsions and deaths in any of the groups. The result is presented in the table 5.

Table 5: *Effect of acepromazine on amphetamine induced aggregation toxicity in mice pretreated with doramectin.*

Groups (n=6)	Piloerection	Fighting behaviour	Convulsions	No. of dead
Control 1	Present	Present	Nil	Nil
Acepromazine treated (2 mg/kg b.wt. ip)				
Control 2	Absent	Absent	Nil	Nil
Test 1	Absent	Absent	Nil	Nil
Test 2	Absent	Absent	Nil	Nil

4. Discussion

Most of the CNS acting drugs influence the locomotor activities in human and animals, which include sedatives, tranquillizers, muscle relaxants, etc. Locomotor activity can be broadly classified into spontaneous locomotor activity (SLA) and forced locomotor activity (FLA), which are measured using various types of activity cages such as photoactometer, rota rod, traction wire, etc.

In the present study efforts were made to assess the pharmacologic effect of an endectoparaciticide, doramectine, on the prototype tranquillizer, acepromazine, in mice by employing various tests. When the result of slow locomotor activity was analyzed (Table 1), it was found that doramectin pretreatment had no significant effect on the depressant action of acepromazine. Till 5th minute there was no difference between the three groups of acepromozine treatment, after which the mice in the groups pre-treated with doramectin showed less SLA but it was non-significant.

Forced locomotor activity (FLA) was tested by using rota rod test (Table 2). The results again indicate no significant effect of doramectin pretreatment on the activity of acepromazine especially at slow speed and medium speed. At high speed of rota rod the doramectin treated mice took observably more time to show fall off but it was statistically not significant. This indicates that the action of acepromazine was attenuated to some extent by doramectin. Thus the observations for acepromazine with respect to FLA are in contrast with SLA, the plausible reason for which could not be found.

In the traction wire test (Table 3), the doramectin 600 μg/kg treated group took significantly more time (47.50±6.19 sec) to show fall from the traction wire. This also indicates that the action of acepromazine was attenuated to some extent by doramectin (at higher dose).

In this study, conditioned response avoidance test was carried out in mice in the pole climbing apparatus described with an electrified grid and light, buzzer arrangement. The study revealed that the action of acepromazine in conditioned response avoidance test was unaffected in both control and doramectin treated groups. This unaltered efficacy of anxiolytic drugs suggests that doramectin does not possess the characteristics of an anxiolytic drug as reported by Spinosa et al. (2000).

Amphetamine induced aggregation toxicity test was conducted to study the protective effect of antipsychotic drug acepromazine in mice. Amphetamine when injected in mice causes excitement which is much more pronounced if the mice are kept together in one cage. Increased stimulation occurring in grouped mice increases the toxicity of amphetamine by many folds. Protection against

the toxicity of amphetamine in grouped mice was studied using acepromazine. Acepromazine at antipsychotic dose was effective in controlling the various characteristics of aggregation toxicity, such as piloerection and aggressiveness, in all the groups including doramectin treated. The observation showed that doramectin did not potentiate the action of acepromozine.

A similar study showed that the drug did not exhibit any anticonvulsant potential at any of the dose (400, 800 and 1600 μg/kg sc) levels studied [10] which correlate our findings on doramectine.

Over all, if the result of all the tests was analyzed, it showed that doramectin did not alter the pharmacologic activity of acepromozine significantly. This could be attributed to the pharmacodynamics of the two drugs. Doramectin acts through GABA receptors and acepromozine acts by blocking the dopamine nerve receptors in the brain [11]. This finding has clinical significance. Especially in surgical patients where acepromazine is used as pre anesthetic and where acepromazine is used as anxiolytic and antiemetic in the patients exposed to doramectin.

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An Experimental Study on Pharmacodynamics Interaction of Doramectin with Anticonvulsants and Anaesthetics in Mice

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Abstract The present study was conducted to examine the pharmacodynamics interactions between doramectin and general anesthetics like diethyl ether and anticonvulsants like phenytoin and diazepam. The methods employed were supramaximal electroshock test (for phenytoin), chemical convulsion test and unconditioned response avoidance test (for diazepam) and different stages of anesthesia and duration of anesthesia (for diethyl ether). In supramaximal electroshock test phenytoin was equally effective in all the groups in controlling grand mal seizures. In chemical convulsion test no response to diazepam was observed in all the groups pretreated with doramectin. And in unconditioned response avoidance test only one mouse showed response to shock in doramectin 200µg/kg group similar to the control 2 group. However, no mouse showed response to shock in doramectin 600 µg/kg groups. No difference was observed in the time taken for exhibiting various stages of anesthesia in different groups in inhalant anesthesia. However the total duration of anesthesia was increased in doramectin (200 µg/kg) treated group (781.67±24.82 sec)) and the value was significantly high in doramectin (600 µg/kg) treated group (861.67±55.69 sec) as compared to 673.33±30.94 sec in control 2. The result suggests that doramectin prolongs the recovery from anesthesia but does not support the anticonvulsant activity.

Keywords *Doramectin, Pharmacodynamics, Diethyl Ether, Diazepam and Phenytoin*

1. Introduction

Avermectins are a group of fermentation products from a strain of Streptomyces avermitilis possessing potent anthelmintic and insecticidal activities. Doramectin is one of the members of this group and is proposed to act on the GABA receptor/chloride ionophore complex. Studies show that doramectin interferes with GABAergic-related behaviours [1, 2]. It is a macrocyclic lactone

disaccharide and is used as a potent broad spectrum veterinary endectocide [3, 4, 5]. It was suggested that doramectin has the pharmacological profile of an anxiolytic/anti-convulsant drug with GABAergic properties [6, 2].

Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system [8]. GABA and its GABA-A receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours [7, 8, 9]. Benzodiazepines (diazepam) are Positive modulators of GABA-A receptors [10, 11, 12, 13].

Phenytoin is an anticonvulsant drug which can be useful in the treatment of epilepsy. The primary site of action appears to be the motor cortex [14] where spread of seizure activity is inhibited. Phenytoin was observed to act through sodium dependent high affinity synaptosomal transport of both glutamate (Glu) and γ-aminobutyric acid (GABA) [15]. Phenytoin tends to stabilize the threshold against hyperexcitability caused by excessive stimulation or environmental changes capable of reducing membrane sodium gradient.

There are reports of preliminary investigations on pharmacodynamics interactions of ivermectin with the CNS acting drugs. But there are very few studies on the pharmacodynamics interactions of doramectin with anticonvulsant drugs like diazepam, phenytoin and inhalant anaesthetic like diethyl ether.

The objective of the present study was to understand the possible pharmacodynamics interaction of doramectin with the anticonvulsant drugs; phenytoin, diazepam and inhalant anaesthetic like diethyl ether.

2. Materials and Methods

2.1. Animals

Male albino mice, weighing 20-25 grams were procured from Central Animal Facility of NIPER, Mohali. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 h light-dark cycle in the departmental animal facility. Animals were given an acclimatization period of seven days before commencing first experiment. Food and water were provided ad libitum. Food was withdrawn 12 h before and during the experiment. Experiments were performed during 8 AM to 12 PM. All experimental protocols were approved by the institutional animal ethics committee (IAEC).

2.2. Drugs

The following drugs were used: doramectin (Dectomax®, Pfizer Inc., USA), diazepam (Calmpose®, Ranbaxy, India), Phenytoin (Phenytoin®, Sigma, USA), Solvent ether (Diethylether® S.D. fine chem. ltd). Doramectin was injected subcutaneously at two doses 200 μg/kg (normal dose) and 600 μg/kg (three times the normal dose). The observations were recorded on the 5th and 6th day since doramectin has a half-life of 1 week.

2.3. Methods

A. *Test for Phenytoin* In this study, the animals was divided into four groups with 6 animals in each group as follows:

Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Phenytoin @ 25 mg / kg b.wt. i.p.
Test 1 -Doramectin 200 μg/kg + Phenytoin @ 25 mg / kg b.wt. i.p.
Test 2 -Doramectin 600 μg/kg + Phenytoin @ 25 mg / kg b.wt. i.p.

Supramaximal Electroshock Test

This test is employed to screen anticonvulsant drugs against grand mal epilepsy, using electroconvulsiometer. In this the mice were applied with pinna (ear) electrode after proper restraining. On the previous day of testing, animals were given sub maximal electrical shock of 6 mA for 0.2 sec duration and those mice, which exhibited extension of hind limbs, were chosen. Next day supramaximal electric shock of 27 mA for 0.2 seconds duration was applied using pinna electrode. The resultant seizures in normal mouse showed a tonic phase of limb flexion for roughly 1.5 seconds followed by full tonic extension for approximately 10 seconds and a few clonic jerks thereafter. This timing was noted in all the mice. The number of deaths due to asphyxia was also noted. The animal after recovery remained in opisthotonus condition and exhibited exaggerated response to stimuli such as clapping or noise. The animals were administered with standard phenytoin 20 min before the actual trials [16].

B*. Test for Diazepam* In this study, twenty four animals were divided into four groups with 6 animals in each group as follows:

Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Diazepam @ 4 mg/kg b.wt. i.p
Test 1 -Doramectin 200 μg/kg + Diazepam @ 4 mg/kg b.wt. i.p.
Test 2 -Doramectin 600 μg/kg + Diazepam @ 4 mg/kg b.wt. i.p.
Two tests were employed for testing the pharmacodynamics interaction of doramectin and diazepam.

Chemical Convulsion Test

In this test, the mice were injected Pentylenetetrazole (@ 70 mg/kg b.wt.) in the scruff of the neck and they were observed for convulsions for the next 15 minutes for clonic convulsions. Administration of Pentylenetetrazole in the scruff of the neck of mouse showed initial tonic phase of limb flexion followed by clonic phase. The clonic convulsions are characteristic of petit mal seizures. Convulsions in which the mouse showed repeated contractions of limbs and body followed by loss of equilibrium was considered as complete clonic convulsion. While short jerky movements were taken as incomplete clonic convulsion. The animals were administered standard Diazepam 20 min before the actual trials [17].

Unconditioned Response Avoidance Test

In this protocol, the mice were subjected directly to shock, without any pre-conditions. This was considered to be the instinctive response to the noxious stimuli, shock. Initial training for 3 days was given to the mice and only those mice that responded to the stimuli by climbing on the pole to avoid the shock were chosen. The standard diazepam was administered 20 minutes prior to trials and the mice were tested for response to shock. Failure of the mice to climb the pole after giving shock was considered as the end point.

C. *Diethyl Ether* Diethyl ether was chosen for the present study since it exhibits all the stages of anesthesia.

In this study, twenty four animals were divided into four groups with 6 animals in each group as follows:

Control 1- No Doramectin + no drug,
Control 2- No Doramectin + Diethyl ether,
Test 1 -Doramectin 200 μg/kg + Diethyl ether,
Test 2 -Doramectin 600 μg/kg + Diethyl ether.

In this test the mice were induced general anesthesia by using diethyl ether in a closed glass observation chamber. The induction time and the recovery time were noted in both controls as well as in the test groups. The time required for transition to various stages of anesthesia was also observed by noting various behavioural signs viz., voluntary excitement (Stage I), involuntary excitement (Stage II) and anesthesia (Stage III) and reflexes such as corneal reflex, palpebral reflex, pedal reflex, etc. to evaluate the depth of anaesthesia. The animals were then placed in supine position and time taken to show righting reflex, three times, was taken as recovery time.

2.3. Statistics Analysis

Results are presented as mean ± standard error of mean (SEM). The level of significance was taken as: $p<0.05$ and $p<0.01$ as significant; $p<0.001$ highly significant.

3. Results

3.1. Supramaximal Electroshock Test (MES)

Supramaximal electroshock technique is used to screen anticonvulsant drugs against grand mal epilepsy, using electroconvulsiometer. The resultant seizures in normal mouse showed a tonic phase of limb flexion for roughly 1.3 seconds followed by full tonic extension for approximately 10 seconds and a few clonic jerks thereafter. This timing was noted in all the animals subjected to MES and presented in table 1. The number of deaths due to respiratory arrest (asphyxia) was also noted.

Table 1: *Effect of phenytoin in mice pretreated with doramectin. (Time taken in seconds)*

Groups (n=6)	Tonic limb flexion	Tonic limb extension	Clonic jerks	Respiration regain	Recovery time
Group 1	1.33 ± 0.21	10.83 ± 0.31	5.67 ± 0.56	11.00 ± 0.52	135.83 ± 16.45
Phenytoin treated (25 mg / kg b.wt. ip)					
Group 2	No response	No response	5.17 ± 0.70	5.33 ± 0.42	88.17 ± 7.04
Group 3	No response	No response	4.50 ± 0.43	7.80 ± 1.06	83.33 ± 6.91
Group 4	No response	No response	4.50 ± 0.43	6.50 ± 0.43	81.17 ± 3.52

On phenytoin treatment the control and doramectin (200 μg/kg) groups showed no response to MES, except for some clonic jerks which lasted for approximately 5 seconds in the mice of all the groups. The time taken to regain respiration in group 2, 3 and 4 were 5.33±0.42, 7.80±1.06 and 6.50±0.43 sec. The recovery time from convulsions was similar in all the groups.

3.2. Chemical Convulsion Test

Pentylenetetrazole induced seizures were employed to screen diazepam against petit mal kind of epilepsy. The observations made are presented in table 2. There was no significant change in the control and doramectin treated mice. Diazepam was able to protect the mice in all the groups from petit mal seizures effectively.

Table 2: *Effect of diazepam against petit mal seizures in mice treated with doramectin*

Groups (n=6)	Tonic phase (min)	No. of convulsions		Recovery time (min)
		Full	Jerky	
Group 1	4.50 ± 0.43	5.67 ± 0.42	6.83 ± 0.79	11.50 ± 0.76
Diazepam treated (4 mg/kg b.wt. ip)				
Group 2	No response	No response	No response	No response
Group 3	No response	No response	No response	No response
Group 4	No response	No response	No response	No response

3.3. Unconditioned Response Avoidance Test

In this protocol, the rats were subjected directly to shock, without any pre-conditions. The mice from all the groups responded to this shock instinctively by climbing on the pole. Diazepam at anxiolytic dose was able to block this unconditioned response. Failure of the mice to climb the pole after giving shock was considered as the end point. The result is presented in table 3. Pole climbing was observed in only one mouse in control group 2 and group 3 (doramectin 200 μg/kg) and no mouse was found to climb the pole in group 4.

Table 3: *Effect of diazepam on unconditioned response test (pole climbing) in rats treated with doramectin. (Value in parenthesis show number of rats responding to electric shock and climbing the pole)*

Groups (n=6)	Response to shock
Group 1	Present
Diazepam treated (4 mg/kg b.wt. ip)	
Group 2	Absent (1)
Group 3	Absent (1)
Group 4	Absent

Altogether, it was observed that the effect of diazepam on unconditioned response was unaltered in doramectin treated mice.

3.4. General Anesthesia

Doramectin treatment did not alter the time taken for exhibiting various stages of anesthesia. However the total duration of anesthesia was increased in doramectin (200 μg/kg) treated group (781.67±24.82 sec)) and the value was significantly high in doramectin (600 μg/kg) treated group (861.67±55.69 sec) as compared to 673.33±30.94 sec in control. The result is presented in the table 4.

Table 4: *Effect of inhalant anesthetics in mice treated with doramectin. (Time in seconds)*

Groups (n=6)	Stage I	Stage II	Stage III	Total duration of Anesthesia
Control 1	125.53± 08.78	291.23± 16.19	352.35± 12.13	668.26± 27.45
Diethyl ether inhalant anesthesia				
Control 2	126.67 ± 14.98	286.67 ± 23.33	346.67 ± 23.33	673.33 ± 30.94
Dora 200	105.00 ± 16.68	280.00 ± 12.11	340.00 ± 12.11	781.67 ± 24.82
Dora 600	103.33 ± 7.71	320.00 ± 9.04	380.00 ± 9.04	861.67 ± 55.69**

** p<0.01, indicates significant difference when compared with the respective control values

4. Discussion

Concurrent administration of doramectin and CNS depressants may result in pharmacodynamics interactions. Perusal of the literature indicates lack of any such systematic studies. Therefore, the present study was envisaged to investigate the possible pharmacodynamics interaction of doramectin treatment with the CNS depressants.

In this study, supramaximal electroshock (MES) technique was used to screen the effect of doramectin pretreatment on phenytoin against grand mal epilepsy, using electroconvulsiometer. From the table 1 it can be said that there was no significant difference in the various observations in different groups. Phenytoin was equally effective in all the groups in controlling MES induced grand mal seizures indicating no pharmacodynamics interaction.

There are contrary views on the pharmacodynamics of phenytoin [18]. Several reports have described that phenytoin causes drug-induced elevations of threshold for activation of the sodium action potential or partial blockade of sodium influx into neurons [19, 20, 21, 22, 23, 24]. There are other reports that say that phenytoin can interfere with voltage-dependent calcium entry [25, 26, 21]. In another report [15] phenytoin was observed to inhibit competitively the sodium dependent high affinity synaptosomal transport of both glutamate (Glu) and γ-aminobutyric acid (GABA). This contrasted with previous reports; the uptakes of glutamate and GABA were enhanced by phenytoin. In another study, [27] Segal and Douglas showed that the anticonvulsant phenytoin diminished late sodium channel openings underlying epileptiform activity.

In our study the results show that there was no interaction between phenytoin and doramectin. This could be reasoned out to the contrary pharmacodynamics of the two drugs. Further study is required to assess the pharmacodynamic interaction of phenytoin with doramectin.

While evaluating the pharamcodynamic interaction between doramectin and diazepam, in both chemical convulsion test and Unconditioned response avoidance test, it was found that there was no significant difference in control and doramectin treated groups. It can be inferred here also that doramectin treatment did not affect the anticonvulsant action of diazepam. Again this was a contrasting result that we have got. Because, benzodiazepines (diazepam) are known to be positive modulators of GABA-A receptors [10, 11, 12, 13, 28]. This could be due to different site of action of doramectin from that of diazepam [6].

There is a report that states the biphasic mechanism of action of diazepam [29] which is not seen in doramectin. Again a contrasting finding in the mechanism of action of diazepam by Bratati and Arvid [30]. The results of the study carried by these two scientists do not support the hypothesis that benzodiazepines act by enhancing GABAergic transmission. It rather suggests that benzodiazepines exert an inhibitory action on transmitter synthesis and utilization at the synaptic level, which is not necessarily bearing any direct relationship to GABA.

Previous studies by Spinosa and coworkers reported that doramectin treatment protected animals from convulsant effect of picrotoxin. They further reported that doramectin has a pharmacological profile of an anxiolytic/anticonvulsant drug with GABAergic properties. In a similar study by the same workers (Spinosa et al) on ivermectin, a structural analog of doramectin, reported that ivermectin protected the animals from convulsant effects of pentylenetetrazole but not from those of picrotoxin. They suggested that the lack of effects on seizures induced by picrotoxin could be due to different site of action of ivermectin from that of benzodiazepine drugs, such as diazepam.

There are contrasting reports on the mechanism of action of doramectin (avermectins in general) as well. There are reports which state that avermectins act by binding to γ-Aminobutyric acid (GABA) receptor [31, 1, 2, 32]. There are reports stating that avermectins act by binding to Glutamate-gated chloride channels [33, 34, 35]. There are reports which state the antagonistic activity of avermectins at GABA receptors [36]. There are reports stating the dual effects of avermectins on GABA gated chloride channels [37]. These reports could be reasoned out for the difference in the pharmacological action of doramecitn and diazepam.

To further assess the putative CNS depressant activity of doramectin, experiments for general anaesthesia was done. Our study revealed that doramectin treatment did not alter the time taken for exhibiting various stages of anesthesia induced by diethyl ether. However the total duration of anesthesia was increased in doramectin treated groups and the value was significantly high in doramectin (600 μg/kg) treated group. This suggests a potentiating action of doramectin on diethyl ether induced anesthesia. This finding is of clinical significance in surgical cases where inhalant anesthetics are used.

Our findings do not support the anticonvulsant/anxiolytic activity of doramectin in mice. Further study is needed to assess the exact pharmacological action of doramectin and anticonvulsants and the pharmacological interaction between doramectin and anticonvulsants at different multiple doses.

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Pericarditis in Bovines- A Review

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Abstract Pericarditis is the most common pericardial disease of cattle. It is an inflammation of the pericardium that results in accumulation of pericardial fluid within the pericardial sac. In cattle, it is mostly caused by the perforation of the pericardial sac by an infected foreign body through the reticulum. Clinical signs of pericarditis are tachycardia, muffled heart sounds, absence of lung sound in the ventral thorax, asynchronous abnormal heart sounds. Distension of the jugular veins and pulsation and submandibular, brisket and ventral abdominal edema are usually present. There is leukocytosis with shift to left and hyperfibrinogenaemia (indicating inflammation).The condition is difficult to diagnose only on the basis of clinical signs. However, imaging techniques like radiography and ultrasonography can be of high diagnostic value in detecting the condition. Treatment is usually not rewarding, but the disease can be prevented to a large extent by the proper managemental practices like feeding of magnets, preventing access to potential and non-potential foreign bodies in the feed and fodder of the animals.

Keywords *Pericarditis, Cattle, Ultrasonography*

1. Introduction

The heart is partially surrounded by a serous membrane called pericardium and it creates a closed cavity called pericardial space that contains small amount of fluid for lubrication [1]. The pericardium is divided into an outer fibrous part and an inner serous part. The fibrous pericardium is a thin sac that covers most of the heart. The outer surface of the fibrous pericardium is covered by the pericardial mediastinal pleura which becomes sternopericardial ligament at the cardiac apex. The base of fibrous pericardium continues onto great arteries and veins that leave and enter the heart. The serous pericardium forms a continuous covering of the heart and inner surface of the fibrous pericardium. The parietal layer is a sheet of continuous mesothelial cells fused to the fibrous pericardium. The visceral layer covers the heart and forms the epicardium. The pericardial cavity is the space between the parietal and visceral layers of the serous pericardium.

Pericarditis is inflammation of the pericardium with accumulation of fluid or exudate between the visceral and parietal pericardium [2]. It is the most common pericardial disorder in cattle [3]. In cattle, it is often attributable to a reticular foreign body that has penetrated the reticular wall, diaphragm and pericardial sac. It is associated with progressive disturbances in heart function and almost always results in death [4]. It is acute, subacute or chronic inflammation of the pericardium due to penetration of pericardium by sharp foreign body. Since distance from the reticulum to the pericardium is only a few cms, sharp contaminated foreign body can easily pierce the diaphragm and enter the pericardium. Rarely, traumatic pericarditis may be caused by the penetration of the wire through the skin, with subsequent migration into the sternebrae and pericardial sac [5]. Krishnamurthy et al reported a case of traumatic reticulo-pericarditis in a cross bred cow caused by penetration of an 8 cm pointed coconut broomstick [6]. Pericarditis attributable to haematogenous spread of infectious diseases (such as colibacillosis, pasteurellosis, salmonellosis and anaerobic infections) is much less common and is usually masked by signs of septicemia [7]. Idiopathic hemorrhagic pericarditis has been recorded as the less common cause of pericardial effusions in cattle with good prognosis [8, 9].

There are three general forms of pericarditis, effusive, fibrinous and constrictive although combination of three can also occur. Effusive pericarditis is characterized by accumulation of a protein rich fluid within the pericardial sac. Subsequent fibrin deposition will lead to fibrinous pericarditis and if fibrin within the pericardial sac matures to fibrinous tissue and fibrosis of the pericardium or epicardium then constrictive pericarditis will result [10]. The incidence is more in cattle and buffaloes than in camels. The incidence of traumatic pericarditis is found more in pregnant or recently parturated animals, due to increased intraabdominal pressure pushes the foreign body towards the thorax. Pericardium may be pierced at one or more places and in few cases; the object may either pierce the myocardium or may come out through the thoracic wall. Sometimes the object may fall back into the reticulum. Trauma to pericardium causes inflammation and exudates continue to accumulate in the pericardial sac. Adhesions between epicardium and pericardium and fluid accumulation in pericardial sac, impairs the ability of the heart to act as a pump and shows signs of congestive heart failure. Right-sided heart failure is common than left sided, as right ventricular wall is thinner than left ventricular wall. Absorptions of toxins lead to toxemia. Death after foreign body penetration of pericardium is usually caused by progressive fibrosing constrictive pericarditis and epicarditis, cardiac tamponade from perfora of the coronary artery has also been found [11].

2. Materials and Methods

2.1. Diagnosis

The diagnosis of the traumatic pericarditis is based on clinical, haematobiochemical, radiographic, ultrasonographic and pericardiocentesis findings.

A. *Clinical Signs* The clinical presentation of pericarditis can vary, depending upon the volume and rate of development of the pericardial effusions and cause. A decrease in appetite and productivity may be the non-specific indicators heart disease (Gonzalez et al). Clinical signs include venous distension and pulsation, tachycardia, and bilaterally muffled heart sounds [12, 13] edema of jaw dewlap and ventral abdominal region extending up to the udder, aorexia, drop in milk yield, reluctance to walk or walk with short steps and with stiff gait, pyrexia, increased pulse rate, abdominal respirations, arched back and abducted elbows. Diarrhea or scant feces is often present [14]. Pinching of withers to depress the back or deep palpation of the ventral abdominal wall behind the xiphoid sternum commonly elicits a painful grunt. Mucous membrane may be congested and have a prolonged capillary refill time. In severe cases, there may be even edema of the conjunctiva with grape like masses of edematous conjunctiva hanging over the eyelids. Arterial pulses are weak. Distension of jugular veins may be attributable to right-sided cardiac insufficiency, the most important cause of which is traumatic pericarditis, valvular endocarditis, cardiomyopathy and cardiac

lymphosarcoma [15, 16, 7, 17]. The clinical signs of jugular engorgement and oedema of the brisket and ventral abdominal wall may also be seen in cases of pseudopericarditis. Pressure caused by tumours, abscesses, Echinococcus cysts, swollen lymph nodes resulting from tuberculosis and leukosis, one-sided pleuritis and diaphragmatic hernia to the base of cranial and caudal vena cava returning blood to the heart have been reported to cause pseudopericarditis in cattle. These mediastinal lymph nodes may also be swollen due to theileriosis and cause pressure on the vena cava [18] and consequently mimics the symptoms of pericarditis.

The most consistent findings on auscultation are tachycardia, muffling of heart sounds, and absence of lung sounds in the ventral thorax. Dorsally the lung sounds are louder than normal [2]. The severity of tachycardia depends primarily on the degree of compression of the heart by pericardial effusion as in cows with idiopathic pericarditis, pericardiocentesis with removal of the fluid results in an immediate decrease in heart rate and cure [8]. The heart sounds are muffled because of pericardial effusion and fibrinous changes in the pericardial sac. The presence of characteristic asynchronous abnormal heart sounds depend on the type of lesions [7]. When the changes are predominantly fibrinous, the sounds are of a rubbing, squeaking or scratching in nature. The predominance of fluid, there are splashing or gurgling sounds, which vary continuously in pitch, loudness, duration and point of maximal intensity. Absence of edema and jugular vein distension does not rule out pericarditis because pericardial fluid can drain into the reticulum via a patent foreign body tract [7]. The respiratory rate is often elevated because of cardiac insufficiency or direct involvement of the lungs. The ruminal motility is usually reduced or absent [4]. On auscultation, initially pericardial friction sounds are heard as parietal and visceral layers rub against each other. In later stages, sounds become muffled due to accumulation of more fluid in pericardial sac. There will be upward and backward displacement of the heart in extreme cases making cardiac sounds audible at the level of shoulder. If the animals survive this advanced stage of pericarditis, it may lead to chronic form called as constrictive pericarditis where heart sounds are muffled due to adhesions between epicardium and pericardium. There will be no pulmonary or limb edema. Effect will be more on thin walled right ventricle than on thick walled left ventricle, so blood pools in systemic circulation. Pleural effusions without cardiac disease must be considered in cattle with muffled heart sounds [19]. Auscultation of muffled heart sounds has high specificity and likelihood ratio for detecting cases of pericarditis [3]. Enlargement of liver may be detectable by palpation behind the upper part of the right costal arch in the cranial part of the right paralumbar fossa [17].

B. *Clinical Pathology* On hematological examination, a pronounced leukocytosis, with a total leucocytes count of 16 000-30 000/µL with shift to left accompanied with neutrophilia and eosinopenia is usual. In the other forms of pericarditis, changes in the blood depend upon the other lesions present and on the causative agent [17]. The glutaraldehyde test, a nonspecific indicator of inflammation due to elevation of fibrinogen and globulin, is an important diagnostic tool because the clotting time is shorter than normal in >90% of cattle [19]. The test can be used to differentiate inflammatory and non-inflammatory right-sided cardiac insufficiency. Hyperfibrinogenaemia, a sensitive indicator of inflammation is also common finding [20, 21, 22]. Hemoconcentration may be present if the animal is dehydrated or toxemic. The activities of γ-glutamyltransferase (γ-GT) and aspartate aminotransferase (ASAT) and the serum concentration of bilirubin are increased, indicating hepatic congestion. Cardiac troponin proteins, which are usually present in blood either at very low concentrations or below the limit of detection of most assays, are released into the circulation in cows with pericarditis [23] hence, may be of value in determining the degree of heart damage in cows with traumaticreticuloperitonitis [24]. Electrolyte concentrations are usually normal, but serum calcium and potassium concentrations may be low because of anorexia [2].

Electrocardiography can aid in the diagnosis. The ECG changes most commonly associated with peircarditis in large animals are decreased amplitude or QRS complex (less than 1.5 mV in the base-apex lead), electric alternans (altered configuration of the P, QRS, or T complexes on a regular basis)

and ST segment elevation or slurring [25, 26, 27]. Removal of large volumes of pericardial fluid does not usually result in immediate changes in QRS amplitude [17]. In a study conducted by McGuirk et al it was noted that persistence of atrial fibrillation was associated with poor performance and progressive cardiac disease, whereas conversion to normal sinus rhythm uniformly led to clinical improvement [28].

C. *Radiography* There is relatively few studies on radiography in cattle with traumatic pericarditis [29, 5, 30, 19, 4]. In cattle, traumatic pericarditis usually results in the gas and fluid accumulation in the pericardium which is visible radiographically and is relatively specific for this disease [2]. In the early stages of traumatic pericarditis, good thoracic detail is evident radiographically while poor differentiation of thoracic structures is evident at progressive stages [30]. Partington and Biller [31] examined 115 consecutively acquired bovine cranial abdominal radiographs and found standing lateral abdominal radiographs to be a valuable tool for the diagnosis of cranial abdominal disorders. The sensitivity and specificity of radiography in detecting traumatic reticuloperitonitis and pericarditis was 83% and 90% respectively. A metallic foreign is usually detected radiographically in the cranial reticulum or caudal thorax in cattle with traumatic pericarditis. Radiographic changes may not be detected in early or uncomplicated pericarditis and if fluid accumulation is large and there is concurrent pleural effusion, are indistinguishable from pleuritis. However, it is difficult to distinguish between pleural and pericardial effusion on the basis of radiography [32, 13]. Radiodense foreign bodies are usually pieces of wire or nails, although other metallic foreign bodies are sometimes seen on the radiograph [4]. Traumatic pericarditis cannot however be ruled out if a foreign body is not seen as thick radiodense adhesions may obscure a foreign body from view or the foreign body may have migrated back into the reticulum, where it may be seen lying freely in the organ or penetrating the reticular wall. Occasionally the offending foreign body attaches to a magnet administered after the damage has occurred. Latero lateral radiographic views are taken of the caudoventral thorax and reticulum with the animal standing (Braun) [4] or right or left lateral radiographs of the cardiac and reticular areas with the animals in dorsal or lateral recumbency are indicated to know the presence and position of foreign body. The cardiac shadow may increase with disappearance of phrenico-cardiac-pulmonary triangle in positive cases [33].

D. *Ultrasonography* Reasonable cardiac images can be obtained with 3.5- to 5-MHz probes for the diagnosis of common conditions. Linear probes are limited to scanning in the vertical plane, but much information can still be acquired. The heart is imaged in the third to sixth intercostal spaces. The ipsilateral foreleg must be positioned forward to allow access to the more cranial spaces. The most common cardiac conditions amenable to diagnosis by generalist ultrasonographers are pericardial disorders, valvular endocarditis, and ventricular septal defects. Pericardial effusions should not be confused with bilateral pleuritis in which anomalies of the pleural space and the lung parenchyma can also be found [34]. Ultrasonography is the method of choice for imaging and characterizing pericardial effusion. Pericardial effusion results in separation of the pericardium from the epicardium, and is most evident over the ventricles. Affected cattle usually have a large amount of hypoechogenic to echogenic pericardial fluid and echogenic deposits and strands of fibrin may be seen on the epicardium. Sometimes strands of fibrin are seen floating in the fluid between the epicardium and pericardium [5, 13, 35, 4]. Abscesses are rarely seen within the pericardial sac. The pericardial layer which is not seen in healthy animals is typically seen as a thick echoic membrane surrounding the heart. The cardiac ventricles are moderately to severely compress depending on the amount of fluid. The lungs are compressed and displaced medially and dorsally [4]. Bright hyperechoic pinpoint echoes representing free gas are often imaged in cattle with pericarditis. Gas in the pericardium may limit the ability to obtain an echocardiographic evaluation of all cardiac structures [2]. In idiopathic pericarditis, the effusion has been reported to be anechoic to hypoechoic, with fibrin adhered to the epicardium in most cases. More echogenic pericardial effusions are seen in animals with septic pericarditis [36]. Ultrasonography of the abdomen usually reveals reticular changes typical of traumatic reticuloperitonitis, such as reduced motility and echogenic deposits. Frequently there is

moderate to severe ascites attributable to cardiac insufficiency. Caudal vena cava often appears dilated and appears round to oval instead of triangular in cross section [4]. Echocardiography permits the differentiation of effusive and fibrinous pericarditis [37, 8]. Echocardiography is useful to confirm the suspicion of pericarditis, to observe the impact of pericardial effusions on the cardiac chambers or function, to differentiate pericardial and pleural effusion and to choose the optimal site of the pericardiocentesis [35].

E. *Pericardal Fluid Analysis* Site for Pericardiocentesis may be selected echocardiographically. Pericardial fluid may be sampled by centesis with a 10 cm 18 gauge needle over the site of maximum audibility of the heart sound, usually the 4th or 5th intercostal space on the left side. Smell of the pericardial fluid reminiscent of retained placenta and toxic metritis is sufficiently diagnostic in cattle with traumatic pericarditis [17]. Protein concentration of greater than 3.5 g/dL and an elevated WBC count of greater than 2500/µL with urine colored to slightly blood tinged, foamy, and foul smelling pericardial fluid in cattle is characteristic of pericarditis [2]. The fluid represents an inflammatory response and may be submitted for bacteriological examination. Fluid analysis and bacterial and viral culture results vary according to the aetiological agent involved. A mixed population of Gram positive and Gram negative aerobic and anaerobic organisms (gastrointestinal flora) [2] sensitive to cephalexin, gentamicin, cotrimethoxazole, tetracycline and erythromycin is usually present [38, 39]. Pericardiocentesis is not without risk. Potential complications include pneumothorax, fatal arrhythmia, leakage of pericardial material into the thorax, resulting in pleuritis and cardiac puncture leading to hemorrhage or death [12]. In chronic cases, only small amounts may be present and a sample may not be obtainable

2.2 Treatment

Treatment of pericarditis in cattle is often unrewarding and usually is addressed toward salvage or short term survival to calving. Treatment should be attempted only in a valuable animal or in an animal carrying a high value embryo. Diuretics are effective in eliminating the severity of peripheral edema; they further reduce venous return and preload in animals with pericarditis [2]. Medical therapy with systemic antibiotics and drainage of the pericardial sac rarely, if ever, permanently cures affected cattle. Therefore most therapeutic efforts have been surgical [12]. Various case reports have described few cattle with a positive outcome and a long term survival [40, 41, 42, 43, 44, 45, 46, 47, 48, 6, 49, 50]. Thoracotomy and pericardiectomy or pericardiotomy have been performed in many fashions in an effort to provide drainage, search for the foreign body, and prevent fluid or later constrictive damage to the heart.

Following thoracotomy, pericardiotomy or pericardiectomy is done. Pericardiotomy involves incising the pericardium and draining the fluid. The foreign body if present is removed and the pericardial cavity is thoroughly irrigated with sterile isotonic saline solution containing antibiotics. An indewelling pericardial drain to allow twice-daily lavage and drainage and instillation of antibiotics may be performed till there is no discharge from the pericardial sac.

Pericardiectomy is indicated in cases of constrictive pericarditis where the mediastinal adhesions are thick and the rigid pericardium and epicardium may interfere with the cardiac filing. Complete removal of pericardium is difficult due to its attachment at the thin walled atria and great vessels. Therefore, partial pericardiectomy is performed by removal of pericardium from the ventricles [33]. However, the surgical procedure risk is prolonged, expensive and of considerable risk [2].

A. *Prognosis* The prognosis of the case varies with the etiological agent. Pericardial disease is usually associated with traumatic reticulopericarditis, but may be caused by neoplastic infiltration (lymphosarcoma) or hematogenous seeding, or by way of local extension. These disorders typically carry a poor prognosis and treatment is thus avoided. There is more favorable prognosis for the

treatment of idiopathic effusive pericarditis reported in cattle and horses with the use of pericardiocentesis, pericardial lavage, and corticosteroids and NSAIDs [37, 9, 8]. Prognosis has been reported to better for cattle in good body condition, with relatively normal appetite, able to ambulate and less than 5 years old [51].

B. *Necropsy Findings* Gross postmortem examination in acute cases shows distention of pericardial sac with foul-smelling grayish fluid containing flakes of fibrin. There may be organization of the fibrinous exudate and serous surface of the sac (epicardium) may be covered with heavy deposits of fibrin. This fibrin frequently gives the appearance of "scrambled eggs". In chronic cases, the pericardial sac is grossly thickened and fused with the pericardium by strong fibrinous adhesions surrounding loculi of varying size which contain pus or straw colored fluid [52, 17, 12, 2]. The causative foreign body can usually be found provided that it has not been previously removed via a rumenotomy [19, 53]. However, sometimes due to extensive adhesions retrieval of the foreign body is not possible even though it has been visualized on radiographs [19].

D. *Prevention and Control* Traumatic pericarditis in cattle can be prevented by routine administration of magnets to heifers at the time of pregnancy diagnosis [2]. Following oral administration, most magnets do not enter the reticulum directly, but are first deposited in the cranial sac of the rumen before entering the reticulum following ruminal contractions [54]. Cattle should be kept away from construction sites and crop fields should be monitored for metal debris. The processed feed can be passed over magnets to recover any magnetic foreign bodies prior to being fed to cattle.

3. Conclusion

The findings of the clinical examination alone do not always allow a definitive diagnosis of traumatic Pericarditis as the typical signs such as tachycardia, muffled heart sound, distension of the jugular veins and edema may not be present in every case. In doubtful cases, radiography and ultrasonography of the heart and reticulum are indicated. Radiography detects metallic foreign bodies and their location within the abdomen or thorax. With ultrasonography, effusion in the thorax, pericardium and abdomen can be detected and characterized. Treatment is usually not rewarding. However, the disease may be prevented by proper managemental practices.

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Seasonal Effects on Estrus Behaviours in Dairy Cattles

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Abstract The present investigation was carried out to evaluate the effects of environmental factors on reproductive performance of bovine (*Bos taurus)* during summer, winter and spring for one year. The study was performed on free moving animals under natural condition during the two consecutive estrus periods by visual observation. The duration of estrus, numbers of mounts were observed. There was a seasonal effect on the duration of estrus; estrus was longer ($P<0.001$) in summer as compared to those of winter and spring. In addition, cows were mounted more times in estrus during winter than in summer and spring. The results of this study revealed that season influences on estrus behaviour of cows.

Keywords *Agro Meteorology, Behaviours, Cow, Environmental Factors, Estrus*

1. Introduction

Environmental factors such as high temperature, humidity and rainfall lead to decrease in cattle's estrus activity [4, 5, 18]. Estrus behaviour was greater in dairy cows observed twice daily when ambient temperature was less than 77°F (25°C) compared with the temperature above 86°F (30°C) [9, 10]. Laboratory studies clearly demonstrated that effects of extreme ambient temperature and environmental control [14, 17] on milk production of dairy cows. Climatological interrelationships have been defined to predict the loss of milk production during summer within some geographical areas of the US [11]. Seasonal depressions of fertility due to heat stress in the bovine are attributable primarily to the female [6].

Furthermore, Ulberg & Burfening, (1967) reported that pregnancy rates declined from 61% to 45% as rectal temperature increased 1ºC at 12h post insemination. The poor reproductive performance associated with thermal stress may be due to high temperature acting directly on the developing embryo and/or through a maternal endocrine imbalance [2]. It is generally noted that the changing temperature which has some relationship with estrogen plays a significant role in maintaining the

body temperature in many mammals. The increased body temperature or compensatory changes in blood flow may indirectly affect hypothalamus by involving changes in body metabolism. In females, the heat stress during the first week of pregnancy results in higher embryo mortality and subsequent abortions [24].

Dairy animals in large operations are housed in facilities, which leave them more exposed to the elements. Both high and low temperatures can affect heat detection. Warm temperatures (>80 F) shorten the estrus period and reduce the expression of heat, which results in decreased heat detection efficiency. Cold temperatures (<0 F) can also have similar effects [8]. In addition, cold temperatures during night time can cause a shift to greater estrus expression during warmer daylight hours. Several reviews have been published on the influence of season and temperature on reproductive performance [22, 13]. However, there is a sparseness of information regarding the environmental conditions, which control reproductive efficiency [15]. It has been noticed that the failure to detect heat can be attributed to about 10% through cow problems and 90% through management problems. Management factors such as frequency of milking, movement of cows, feeding, and types of footing surface may alter estrus behaviour [16, 1, 3].

The objectives of the present study are to analyze the effects of environmental temperature on reproductive performance and to consider certain physiological and hormonal factors responsive to environmental and management conditions that may in turn affect the cow's performance, and to document, under the benefits of environmental factors, practical conditions in response to reproductive performance. Factors affecting estrus behavior in bovine, *Bos taurus,* have not been well documented. Hence, the present investigations were carried out to evaluate the estrus behaviour, management and environmental factors that affect the estrus, and to improve heat detection rates in various seasonal conditions.

2. Materials and Methods

2.1. Animals

Estrus behaviours were determined during two consecutive estrous cycles in summer, (April, May, June, July n=17), winter (August, September, October, November n=20) and spring (December, January, February, March, n=17). Estrus behaviour was characterized by the number of mounts and the duration of estrus. Seasonal effects and estrus behaviours were observed in the exotic cattle-breeding center, Eachenkottai, Tanjore District, Tamil Nadu, India.

The metrological data viz., atmospheric temperature (ºC), rainfall (mm), humidity (%), sunshine (hours/day) and evaporation were collected from Tamil Nadu Rice Research Institute, Tamil Nadu Agricultural University, Aduthurai, India. Seasonal influences on estrous behaviour were noted in bovine, *Bos taurus*. Cows were observed in each season in a 30-acre field with both natural and artificial shade. There were no ponds in the field, and cows received water from a metal tank.

2.2. Estrus Detection

During the summer, winter and spring of the first year, cows were observed twice daily for 30 min for detection of estrus. The duration of estrus and the number of mounts received per hour of estrus were recorded. The onset of estrus was determined as the first of two mounts received within 4h.The end of estrus was confirmed while the last mount was received, with a mount 4 hour before, and no mounts during the next 12h. The number of mounts received was the number of times a cow was mounted by herd mates. The data were analyzed as mounts received per hour of estrus.

3. Results

3.1. Estrus Behaviour

In the present study, the duration of estrus and the number of mounts were visually observed for one year. The duration of the estrous cycle was about 19 days in cows within a normal range of 18-21 days and 20 days in heifers. There was a seasonal effect on the duration of estrus, in cows. The duration of estrus was longer in summer (21.02±0.35) than in winter (18.73±0.18) and in spring (18.04±0.19). The cows were mounted more times per estrus in winter (61.52±0.44) than in summer (49.51±0.28) and in spring (39.69±0.43). During all seasons, the numbers of mounts was more during estrus than in all other periods. The maximum duration of estrus in summer was 21.02 hr. (Table-3 and 3.1).

3.2. Agro Meteorology Weather Data

In India generally, December to February is the coolest period and April to June is hot summer. The maximum temperature of 28.2 ºC was noted during December 2004, whereas in May it reached the highest level of 37.7 ºC. Likewise, the minimum temperature of 20.9 ºC was noted during January 2004, whereas the highest level of 26 ºC was during July, 2004. The minimum rainfall was 14.6 (Mm) during January 2005, but in October it reached the highest level of 213.4 Mm. The minimum relative humidity (RH) during June (2004) was 76% whereas in January & February, 2005, it reached the highest level of 94%. The minimum wind velocity was 3.4 km/h during November, 2004 whereas in July it reached a high-level i.e.11.0 km/h. The data on the other metrological features of study area are given in table -1 and 2.

Table 1: *Metrological Features of the Study Area during January to December 2004*

Month	Temperature		Relative Humidity (%)	Wind Velocity (km/h)	Sunshine (hours)	Evaporation	Rainfall (mm)	RD
	Max. ºC	Min. ºC						
Jan	29.1	20.9	94	4.8	5.5	2.2	13.0	1
Feb	32.8	20.5	95	3.5	8.8	3.5	0	0
March	34.8	22.1	92	3.9	8.3	3.9	0	0
April	35.5	24.4	84	3.9	6.8	3.7	43.2	3
May	37.7	26.0	79	8.2	8.5	5.1	97.4	3
June	35.5	25.8	76	10.9	5.6	4.4	27.2	3
July	35.4	26.0	79	11.0	6.0	4.8	48.4	3
Aug	34.4	25.2	80	8.7	7.1	4.8	60.8	3
Sept	34.1	24.4	85	5.0	6.7	3.7	102.8	6
Oct	31.9	24.5	88	4.3	5.2	2.7	213.4	11
Nov	30.2	23.4	94	3.4	4.5	1.8	172.6	12
Dec	28.2	21.2	93	5.1	4.8	1.6	157.2	10

Table 2: *Metrological Features of the Study Area during January to December 2005*

Month	Temperature		Relative Humidity (%)	Wind Velocity (km/h)	Sun Shine (hours)	Evaporation	Rainfall (mm)	RD
	Max. ºC	Min. ºC						
Jan	29.5	20.9	95	4.9	6.0	2.3	14.6	2
Feb	30.8	21.0	95	4.3	7.5	2.4	205.2	4
March	34.3	22.4	94	3.8	8.3	3.1	-	-
April	36.6	25.7	87	4.7	9.8	3.8	-	-
May	38.0	26.1	82	7.8	8.0	3.7	46.2	2
June	35.7	25.4	81	11.3	6.6	3.2	121.0	4
July	35.7	25.7	82	11.4	7.1	3.3	73.0	-
Aug	34.4	25.1	79	9.7	6.5	3.9	82.0	-
Sept	36.4	24.7	85	5.2	8.1	5.6	14.2	1
Oct	32.4	24.0	91	2.5	5.0	2.6	416.8	11
Nov	30.0	22.6	94	3.8	4.7	1.9	167.8	8
Dec	29.9	20.5	93	4.8	6.1	2.6	60.6	2

Table 3: *Seasonal Effects and Estrus Behaviours*

Nature of Behaviour	Summer	Winter	Spring
Duration of estrus	21.02 ±0.35	18.73±0.18	18.04±0.19
No of mounts	49.51±0.28	61.52±0.44	39.69±0.43

Values are mean ± S.E of six observations

Table 3.1: *Analysis of Variance (ANOVA) with Post Hoc Comparison (One-Way)*

ANOVA						
		Sum of Squares	df	Mean Squares	F	Sig
VAR 00001 (Duration of estrus)	Between squares	29.090	2	14.545	44.406	.000**
	Within groups	4.913	15	.328		
	Total	34.003	17			
VAR 00003 (Number of mounts)	Between squares	1435.076	2	717.538	564.537	.000**
	Within groups	19.065	15	1.271		
	Total	1454.141	17			

Level of significance at p <0.001

Means for groups in homogenous subsets are displayed comparison of means using DMRT.
The DMRT test showed that the duration of estrus in summer were found to be significant ($P<0.001$) compared to those of winter and spring season and number of mounts in winter were found to be more significant ($P<0.001$) than in summer and spring season.

4. Discussion

The present study revealed that the average estrous cycle was 19 days in cows, which indicate that the estrous cycle falls in the normal range of 18-21 days. This observation clearly revealed that all the cows selected for behaviour assay exhibited effective estrus behaviour. In fact, dairy cattle have a shorter duration of estrus than beef cows [23]. Dairy cattle may have a shorter duration of estrus and less mounts than beef cows. The duration of estrus in dairy cattle averaged 9.5 h with 10.1 mounts, and the season of the year did not influence behaviour [23]. Dransfield et al., (1998) found an average for duration of estrus was 7.1 h with 8.5 mounts in dairy cows. In the present study, among the duration of estrus in summer, winter and spring, the DMRT test clearly showed that the duration of estrus in summer was found to be more variable ($p<0.001$) than that in winter and spring (Tables-3 and 3.1). These reports clearly indicate that the duration of estrus was higher in summer than in winter and spring.

As far as mounting activity is concerned, cows received more mounts ($P<.05$) in winter than in summer and the duration of behavioral estrus was longer in winter ($P<.005$). In the present study, among the number of mounts in summer, winter and spring, the DMRT test clearly showed that the number of mounts in winter was found to be more ($p<0.001$) than in summer and spring (Table-3 and 3.1). The expression of less mounts and a shorter duration of estrus in dairy cows than in beef cows may be because of environmental factors that differ in production practices for the breeds. Confinement, milking, contact with human, stress, and other factors may influence the estrus behaviour of cattle [21]. Estrus did not commence at a particular time of the day, and the first mount was evenly distributed across 24 h. This is in agreement with mature dairy cattle [7], but differs from beef heifers [19].

The results clearly revealed that the cows were mounted more times during estrus than during the other periods. Saacke et al., (2000) reported that the fertilization rate was greater when dairy cows were inseminated 24 h when compared with 0 and 12 h after the first mount was received, as determined by the heat watch system. Using continues visual observation; Pennington et al., (1985) also found that dairy cows had a longer, less intense estrus in hot weather, with longer intervals between mounts received. Seasonal effects on estrus behaviour may differ among studies due to the breed of cows; climate or management practices [13].

It is believed that abiotic factors such as temperature, humidity, and climate may affect the behaviour of estrus cows and may be influenced by environmental factors [20]. Reproductive problems occur in herds of all sizes, but practical solutions may differ in the cases of larger and smaller units. Detection of estrus is difficult, since the expression of estrus behavior varies between cows and also between the first postpartum and all subsequent cycles within the cows.

Seasonal effects on estrus could be due to climate effects on the willingness of herd mates to mount, rather than to a physiological effect of temperature on the cow in estrus. Environmental temperature and relative humidity can depend on husbandry and housing design and can differ considerably between primary and secondary enclosures [21]. Factors that contribute to variation in temperature and humidity include housing material and construction, use of filter tops, number of animals per cage, forced ventilation of the enclosures, frequency of bedding changes, and bedding type [12].

To minimize the extent and impact of these changes, even under climates of only mild to moderate heat stress, may be necessary. However, the above study concludes that optimal temperature of summer appears to be an effective reproductive carrier.

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