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Study on prevalence of helminth infestation and anthelmintic resistance in calves in semi-arid subtropical region of lower Gangetic plains

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Abstract
This research was focused mainly on "Helminths Infestation and Anthelmintic resistance in calves". The main objective of research was to find out the prevalence of helminth infestation and the status of anthelmintic resistance in the calves in semi-arid subtropical region of lower Gangetic plains in year 2021. We targeted the calves of 0 to 6 months of age. 54 samples were collected, forty calves out of 54 were found to be infected with one or more species of gastrointestinal parasites, the prevalence rate of helminth parasitic infection was calculated at 74.04%. Forty helminths positive calves were randomly divided into four groups, *viz*. control (without any treatment), T_1, T_2 and T_3, each consisting of 10 calves. Each group received different antihelminthic drugs, Fenbendazole, Ivermectin, and Levamisole, respectively. on day 0 and on day 14, the efficacy of these popular anthelmintic drugs was assessed through EPG (Egg Per Gram) and FECRT (Faecal egg count reduction test). The efficacy of anthelmintic drugs was determined through the percentage value of FECRT, which is less than 90%. FECRT values were found to be 58.33%, 65%, and 84.21%, EHA (Egg Hatch Assay) was done to find resistance in helminths against these three anthelmintic drugs. The high prevalence of anthelmintic resistance found in this study indicates that AR is a threat to our livestock production. Sustainable worm control strategies, such as farm and targeted treatment, which prolong the life span of the currently used anthelmintic, need to be implemented as a matter of urgency.

Keywords: Anthelmintic, fenbendazole, levamisole, EPG, FECRT, ivermectin

Introduction
Animal husbandry is an important part of agriculture, which plays a crucial role in the economy of the country. Livestock production is a major earning source for the agricultural sustainability of poor farmers in rural areas, especially when crop production is not a profitable source of income (Khajuria *et al*., 2013; Ahmed *et al*., 2020) [19, 1]. "Drug Resistance" is defined as the ability of worms in a population to survive drug treatment that is generally effective against the same species and stage of infection at the same dose (Sangster, 1999) [20]. In recent years, a rapid increase in both the prevalence and magnitude of anthelmintic resistance occurred (Kaplan *et al*., 2012) [9]. Helminth infestations in animals have adverse effects on their health. Gastrointestinal nematodes are a major problem in animals. Due to helminth infestation, stockbreeders have to suffer a huge financial loss. These parasites affect the cattle's health, which leads to decreased production and average daily gain (ADG), resulting in estimated economic losses of over $2.5 billion a year for cattle producers (Williams and Loyacano, 2001) [26]. The tropical environmental conditions of India are much more favourable to the survival and spread of helminths. Animals get infected with parasites by consuming contaminated soil and grass. Due to helminths infestation in the stomach of milch animals, their performance decreases, milk production falls, and meat animals' weight does not increase. The livestock industry suffers huge economic losses. Severe infection inflicts a substantial impact on milk, meat, and wool production, reduces weight gain by 23-63%; and in 25% of cases, death occurs before weaning (Singh *et al.*, 2015) [21]. The problem of gastrointestinal helminth infection is an important cause of disease and creates significant

threats to health and production losses in the live stock industries throughout the world (Fikru *et al*., 2006) [5]. Gastrointestinal (GI) parasites cause impaired digestion and affect the absorption of minerals, particularly calcium and phosphorus. Parasites become resistant to that drug by giving a low dose of an anthelmintic drug below the recommended dose or by using the same anthelmintic for a long time. Anthelmintic resistance appears in new-born and young animals with greater intensity than in adult animals. Anthelmintic resistance in animals will become a major worldwide barrier to livestock production if ignored, and anthelmintic drugs will be completely ineffective.

Materials and Methods

The present study was conducted in the Department of Veterinary Medicine, Bihar Veterinary College, Bihar Animal Sciences University, Patna. A random sample collection was done from the ILFC and khatals located in and around Patna. A total of 52 calves aged between 0-6 months with suspected helminth infestation were screened by faecal smear examination. The calves which were found to be infested with helminths were subjected to further study. A total of 30, helminth infested calves were randomly divided into three groups, *viz*. T_1, T_2 and T_3, each consisting of 10 calves. The groups T_1, T_2 and T_3 were treated with Fenbendazole @ 10 mg/kg, Ivermectin @ 0.2 mg/kg and Levamisole @ 7.5 mg/kg respectively. One control group, marked as C, comprised of 10 healthy calves, was also formed and no treatment was given. The efficacies of anthelmintics were assessed by FECRT (Faecal egg count reduction test) *in vivo* and anthelmintic resistance was measured by the EHA (Egg hatch assay) protocol *in-vitro*. The EHA was performed in a laboratory at different concentrations of the above noted drugs. Three types of concentrations were prepared: 1 µg/ml, 5 µg/ml and 10 µg/ml for all the three drugs.

FECRT (Faecal egg count reduction test)

$$\text{Percentage reduction in EPG} = \frac{\text{Pre-treatment mean EPG—Post-treatment mean EPG}}{\text{Pre-treatment mean EPG}} \times 100$$

The standard interpretation of the FECRT results in livestock was on the basis of the following criteria: FECRT > 98%, highly effective; FECRT 90- 98%, effective; FECRT 80-89%, moderately effective; and FECRT < 80% insufficiently active or resistance.

Egg per gram: EPG will be determined (pre-treatment) and after 7th day post treatment by modified Mc-Master technique.

Result and Discussion

In this study, 54 samples were collected from in and around Patna. Forty calves out of 54 were found to be infected with one or more species of gastrointestinal parasites, i.e; *Monezia, H. contortus, Cooperia, Trichuris* spp. The prevalence rate of helminth parasitic infection was calculated at 74.04%. Despite the importance of calves for the future, there is a distinct lack of information on the deworming and anthelmintic resistance among gastrointestinal parasites in the study area. High temperatures and humidity promote the survival of the parasite's infective stage and the intermediate host, as do management conditions and deworming practices. Similar finding has been reported by many workers across the country. As per the finding of Kashyap *et al*., (1997) [10], a 40.3% prevalence of gastrointestinal helminths was found in the Malwa region of Madhya Pradesh and Gupta *et al*., (2012) [7] also recorded a 68.93% prevalence in cattle and buffalo in the same locality. The variation in findings with the earlier report might be due to the difference in the number of faecal samples examined, the age of the animal, the geo-climatic condition of the area and the period of study.

Table 1: EPG of different groups at 0 day and 14th day

Sr. No. of calves	Age in months Group				EPG,0 day Group				EPG,14th day Group			
	Tc	T_1	T_2	T_3	Tc	T_1	T_2	T_3	Tc	T_1	T_2	T_3
1	3	2	4	2	100	50	100	50	100	0	50	0
2	1	6	1.5	3	50	250	50	50	0	150	0	0
3	2	3.5	5	4	50	100	150	150	150	0	50	50
4	4	2	2	1.5	150	50	50	50	100	0	0	0
5	3.5	2.5	1	3	100	100	50	100	150	50	0	0
6	2	3	2.5	6	50	50	100	200	100	0	50	50
7	6	4.5	3	2.5	200	150	100	100	150	100	0	0
8	1.5	1.5	5.5	3	10	200	200	50	100	100	0	0
9	5	4	1.5	1	150	100	50	50	200	50	100	0
10	4.5	1	4	5	100	150	150	150	150	50	50	50
Mean EPG					105	120	100	95	120	50	35	15

In the control group (Tc), 10 out of 40 infected calves were not given any medication for treatment. As it can be seen from the table, in calf 2, 4, and 7 of this group, helminth infestation has decreased naturally. However, in other calves of this group, the infestation has increased significantly due to lack of any treatment. The mean EPG of this group has increased from 105 to 120. This clearly indicates that appropriate treatment is needed to fight against helminths infestation or else the level of helminths infestation may rise.

In the first treatment group (T_1) of 10 infected calves, received Fenbendazole for treatment. Looking at the table, it can be observed that after treatment, calf 1, 3, 4 and 6 of this group have become completely free of helminth infestation. Helminth infection has decreased in the remaining 6 calves, but they remain infected. The mean EPG of this group is reduced from 120 to 50.In the majority of studies, it has been found that fenbendazole is insufficiently active. FECR is 58.33% against nematode parasites of calves. This could be due to the frequent use of fenbendazole as an anthelmintic in the study area. Similar observations have been reported by Lacey *et al.*, (1994) [15], who found resistance to fenbendazole on the basis of FECR.

In this group, (T_2) calves were given Ivermectin anthelmintic. Looking at the table, it can be seen that on the 14th day of treatment, calf 2,4,5,7, and 9 of this group have become completely free of helminth infestation. Helminth infestation has decreased in the remaining 5 calves, but they are not infection free. The mean EPG of this group is reduced from 100 to 35. The value of FECR is 65% which shows that resistance has developed in helminth against ivermectin. In this group, (T_2) calves were given ivermectin anthelmintic. A number of studies have pointed out that the AR in gastrointestinal worms Suthland *et al.* (2011) [22]. Ivermectin resistance is particularly more common in Cooperia spp. which is the dose limiting species Geurden *et al.* (2015) [6]. The value of FECR is 65% which shows that resistance has developed in helminth against ivermectin. Kudo *et al. (2014)* [14] found in their research that the range of FECR for ivermectin was 16-87% in 2009 and 24-96% in 2010. Findings by McMahon *et al.* (2013) [17] showed that resistance

to ivermectin was 50% in Northern Ireland. In a similar study, Verssimo *et al.* (2012) [24] reported 54% resistance to ivermectin in Brazil. Waghorn *et al.* (2006) [25] also reported that FECR for Ivermectin was 25%, which shows complete resistance. And according to Van Wyk *et al., in 1999* [23], resistance to ivermectin was 73% in South Africa

In the last group (T_3), calves were given Levamisole as an anthelmintic. Looking at the table, it can be seen that after treatment, 7 calves fully recovered from infection, while 3 calves still have helminth infection. Although after treatment, a significant improvement in the level of infection can be seen in these 3 calves, the fact that they're not getting infection-free was a cause of concern. The mean EPG of this group decreased from 95 to 15, which is significantly lower than the previous one. As a result, the value of FECR has come down to 84.21% which is very close to 90%. This indicates that the level of resistance in helminths against Levamisole is low as compared to other anthelmintic used in this study. In the last group (T_3), calves were given Levamisole as an anthelmintic. The value of FECR is 76% which shows that resistance has developed in helminths against the levamisole. It was found to be most effective against GI worms, as evident from table (3), which clearly states that there was no hatching seen in both 5 µg/ml and 10 µg/ml concentrations of LV. This means that these concentrations can kill larvae of various helminths *in vitro*, and they may also kill them *in vivo*, Keegane *et al.* (2017) [11] showed in their research that resistance to levamisole was 47% in Ireland. In another research, Easwaran *et al.* (2009) [4] reported that the range of FECR was 70-82%, which was quite similar to our findings.

The EHA was performed *in-vitro* using three different concentrations of fenbendazole, i.e. 1 µg/ml (concentration 1), 5 µg/ml (concentration 2) and 10 µg/ml (concentration 3). In treatment with fenbendazole in a 1 µg/ml concentration, 2 eggs were viewed under a microscope and both were found to hatch. In 5 µg/ml concentration, hatching was seen in one egg. On the other hand, in 10 µg/ml concentration, both eggs were found to be unhatched. In conclusion, it can be said that helminths have developed resistance to 1 µg/ml and 5 µg/ml of fenbendazole while sensitivity to 10 µg/ml concentration is still present. The EHA was performed *in-vitro* using three different concentrations of Ivermectin, i.e. 1 µg/ml, 5 µg/ml and 10 µg/ml. In treatment with Ivermectin, in 1 µg/ml concentration, 2 eggs were viewed under a microscope and both were found to hatch. In 5 µg/ml concentration, hatching was seen in one egg while the other remained unhatched. In a 10 µg/ml concentration, both eggs were found to be unhatched. In conclusion, it can be said that helminthes have developed resistance to 1 µg/ml and partial to 5 µg/ml of Ivermectin while sensitivity to 10 µg/ml concentration is still present. The EHA was performed *in-vitro* using three different concentrations of levamisole, i.e. 1 µg/ml, 5 µg/ml and 10 µg/ml. One egg was examined under a microscope after being treated with levamisole at a concentration of 1 µg/ml and found to hatch. In 5 µg/ml concentration, 3 eggs were seen, but hatching wasn't seen in any of the eggs. On the other hand, at 10 µg/ml concentration, both eggs were found to be unhatched. In conclusion, it can be said that helminthes have developed resistance to 1 µg/ml while at 5 µg/ml and 10 µg/ml of levamisole they were sensitive. All the anthelmintic used in 1 µg/ml concentration were found to be resistant during the *in-vitro* egg hatch assay. However, 10 µg/ml concentration of all the drugs was found to be sensitive. 5 µg/ml concentration of IVM shows resistance while levamisole is sensitive. A number of studies have demonstrated AR against GI helminth worldwide, Suthland *et al.* (2011) [22]. IVM resistance is commonly found in *Cooperia spp.* Geurden *et al.* (2015) [6] and similar finding has been observed in present study. Fenbendazole and levamisole resistance has also been reported by Kelleher *et.al* 2020 [12]. However, in present study the LM is comparatively found to be sensitive than the other two drugs. This could be due to less use of said drug in the deworming schedule of livestock in the study region.

The anthelmintic resistances found in this study show that helminth control in calves is urgently needed with the appropriate drugs. There is a dearth of information available for the parasitic control of livestock in the study area and these needs to be examined in order to identify risk factors associated with the development of anthelmintic resistance and take appropriate steps to mitigate these risks. An indicator such as FEC or weight gain should be utilised to help target anthelmintic treatment. In addition, further research is required to be conducted to reduce the AR or manage AR, such as combination therapy or targeted selective treatment, is required.

Table 2: Percentage reduction in EPG of different group before and after treatment

Sr. No. of calves	EPG,0 day				EPG,14th day				% reduction FECR			
	Group				Group				Group			
	Tc	T_1	T_2	T_3	Tc	T_1	T_2	T_3	Tc	T_1	T_2	T_3
1	100	100	100	100	100	50	50	0	0	50	50	100
2	50	250	50	50	0	150	0	0	100	40	100	100
3	50	100	150	200	150	0	50	50	-200	100	67	75
4	150	200	100	100	100	50	50	50	33	75	50	50
5	100	100	200	150	150	50	50	50	-50	50	75	67
6	50	50	100	200	100	0	50	50	-100	100	50	75
7	200	150	100	100	150	100	0	0	25	33	100	100
8	100	200	200	150	100	100	100	50	0	50	50	67
9	150	100	50	50	200	50	0	0	-33	50	100	100
10	100	150	150	150	150	50	100	50	-50	67	33	67
									-14	57	63	76

Table 3: Egg hatch Assay in different anthelmintics *in-vitro*

Observation	Fenbendazole			Ivermectin			Levamisole		
Concentration in (**µg/ml**)	1	5	10	1	5	10	1	5	10
Hatching of eggs	2	1	0	2	1	0	1	0	0
Conclusion	R	R	S	R	R	S	R	S	S

*R= resistant, S=Sensitive

Conclusion

The calves are said to be future stock of livestock. Effective control of helminths is essential for sustainable livestock production. Efficacious helminth control has been dependent on the availability of suitable anthelmintic. The high prevalence of anthelmintic resistance found in this study indicates that anthelmintic resistance is a threat to our livestock production. Sustainable worm control strategies, such as farm and targeted treatment, which prolong the life span of the currently used anthelmintic, need to be implemented as a matter of urgency.

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