In-vivo Acaricidal Efficacy of Aqueous Extract of Azadirachta Indica Leaves in Cattle

R. I. Hatzade (dravihatzade@rediffmail.com)  
Maharashtra Animal and Fishery Sciences University  
https://orcid.org/0000-0002-7990-0081

S. P. Waghmare  
Maharashtra Animal and Fishery Sciences University

A. U. Bihikane  
Maharashtra Animal and Fishery Sciences University

S. W. Kolte  
Maharashtra Animal and Fishery Sciences University

S. V. Kuralkar  
Maharashtra Animal and Fishery Sciences University

S. W. Hajare  
Maharashtra Animal and Fishery Sciences University

R. S. Ingole  
Maharashtra Animal and Fishery Sciences University

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**Abstract**

The 70% (v/v) aqueous extract of *A. indica* leaves evaluated for its efficacy against cattle ticks. The group T1 and T2 were treated with 70% aqueous extract of *A. indica* leaves topically once in a day and twice at 24 hrs interval, respectively. The tick count in 10x10 cm areas on different regions of animal body was carried out on '0' day (before treatment) and 24 hrs, 3rd, 7th, 14th and 28th day after treatment. The per cent efficacy of extract was evaluated on the basis of reduction in tick count with in the observed area on the treated animals. The average tick count in T1 and T2 group was 55.80 ± 11.29, 49.90 ± 11.08, 35.40 ± 7.95, 24.00 ± 5.74, 17.90 ± 7.35, 11.70 ± 2.87 and 107.60 ± 28.12, 97.00 ± 27.85, 83.30 ± 29.75, 70.30 ± 30.51, 62.70 ± 32.97, 18.80 ± 5.05 on day 0 (pre-treatment), 1, 3, 7, 14 and 28, respectively. The tick count was progressively reduced with increase in per cent efficacy after treatment at various intervals in both the groups. The statistical analysis revealed significant variation between different intervals in group T1 with significant reduction in tick count from 7th day (24.00 ± 5.74), 14th day (17.90 ± 7.35) and 28th day (11.70 ± 2.87) post treatment as compared to pre-treatment tick count (55.80 ± 11.29). In T2 group, the average tick count was found non-significant at various treatment intervals, however, it was markedly reduced on 28th day (18.80 ± 5.05) after treatment as compared to pre-treatment tick count (107.60 ± 28.12). Per cent efficacy was higher in T2 group (82.53%) than T1 group (79.03%) on 28th day after treatment. The reduction in tick count might be due to the most prominent phytoconstituent azadirachtin present in *A. indica* leaves. Clinical parameters in all the animals were within normal reference range at various treatment intervals in both the groups. The skin coat becomes smooth and shiny after treatment from 14th day with reduction in tick burden.

**INTRODUCTION**

Many factors affects the health of livestock such as infectious and contagious diseases, poor management practices, nutritional imbalance, ecto and endo parasites etc., which are responsible for severe economic losses to the livestock industry. The major constraint in livestock sector is the ectoparasites mainly ticks infestation which is one of the serious animal health problem in the world that causes substantial economic losses, particularly in tropical and subtropical countries (Colebrook and Wall, 2004; Rodriguez et al., 2011). These ectoparasites can impact the production and health of the animals, either by directly or indirectly causing significant losses in the production of meat, milk, eggs, leathers and in many cases the death of the affected animals (Eskezia and Desta, 2016).

The chemical acaricides are extensively used for the management of ticks and tick borne diseases in tropical and subtropical countries as a short term measure. However, continuous use of chemical acaricides has led to the development of acaricide resistant tick populations throughout India (Sharma et al., 2012; Kumar et al., 2020, Bisht et al., 2021; Fular et al., 2021 and Shakya et al., 2022, toxic residues in livestock products and environmental pollution (Vudhiko et al., 2016 and Choudhary et al., 2018). Keeping this in view, the current situation in India demands a development and promoting affordable, easily available, non-toxic, eco-friendly, effective and safe acaricide formulation for implementation of effective tick control strategies (Solomon et al., 2001). One approach to solving the above challenges is to search and integrate the existing conventional methods of health management and ethno-veterinary practices, particularly the use of medicinal plants.

The plant *Azadirachta indica* (Neem) a fast growing widely distributed indigenous plant has been used for a long in agriculture and medicine (Natarajan et al., 2003). It is regarded as “Village dispensary” in India and its importance is recognized by US National Academy of Sciences and published a report in 1992 entitled ‘Neem-a tree for solving global problems’ (Kirtikar and Basu, 1975; Biswas et al., 2002). Internationally neem has gained importance as all communities have inclined towards the green technology. Neem products are without ill effects on animals and humans and have no residual effect on agricultural produce. This makes neem the best, reliable substitute to hazardous pesticides. Large scale use of neem based pesticides will reduce the demand for chemical pesticides which in turn reduce the load of synthetic chemicals in environment (Girish and Bhat, 2008). The excellent bioactivity of this plant is due to the chemical compounds such as azadirachtin, nimbin, nimbidin and salanin (Rao et al., 1986). Many scientific studies established the importance of the neem tree that it contain chemical compounds to control more than 100 species of insects and microorganisms (Vaideki et al., 2002). On the basis of results of in-vitro acaricidal study and acute dermal toxicity study, the 70% concentration of extract of neem leaves have been evaluated in tick infested cattle. Thus, the present study was undertaken to evaluate the acaricidal activity of aqueous extract of *Azadirachta indica* to evolve effective, cheap, eco-friendly, easily available and safe herbal acaricidal for tick control in cattle.

**MATERIALS AND METHODS**

**Preparation of Leaves Extracts**

Leaves of the plant *Azadirachta indica* were collected from the college campus of Post Graduate Institute of Veterinary and Animal Sciences (PGIVAS), Akola, Maharashtra, India. The collected plant materials were identified and authenticated by the expert botanist, cleaned and shade dried. Dried leaves were then powdered using a mechanical grinder. 100 gm of powder soaked in 500 ml distilled water for 24 hours with vigorous shaking every 3–4 hours and then filtered through muslin cloth to obtain extract as stock solution. Then these extracts assessed for qualitative phytochemical analysis and chromatographic evaluation using standard laboratory methods and high performance thin layer chromatography (HPTLC) technique respectively before they subjected to study their acaricidal potential at various concentration using adult immersion test (AIT) and larval packet test (LPT) against cattle tick *Hyalomma anatolicum*.

**In-vivo evaluation of acaricidal efficacy**

Based on in-vitro acaricidal evaluation and acute dermal toxicity testing, the 70% aqueous extract of *A. indica* leaves was assessed for in-vivo acaricidal efficacy in tick infested cattle. The present research work was carried out in 20 cattle of either sex aged between 1 to 10 years infested with tick count of 5 and above in 10 x 10 cm areas on different regions of animal body from the Goshala, cattle brought to the TVCC of PGIVAS, Akola and villages around Akola. The
selected cattle were not received any kind of acaricide 45 days before the beginning of tick counts, as well as during the study as recommended by Furlong and Martins (2000). All the animals were subjected for thorough physical and clinical examination to rule out systemic illness, if any. Only apparently healthy cattle by history from owner and physical examination were enrolled for this study.

Total 20 selected tick infested animals were divided randomly into 2 equal groups, comprising 10 animals in each group (Table 1). The first group T1 was treated with 70% aqueous extract of A. indica leaves topically once a day. The second group T2 was treated with 70% aqueous extract of A. indica leaves topically twice at 24 hrs interval.

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<th>Table No. 1 In-vivo treatment groups</th>
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Calculation of tick burden
The ticks were counted in 10 x 10 cm areas of different regions on animal body on ‘0’ day and on 24 hrs, 3rd, 7th, 14th and 28th day after treatment with slight modification as suggested by Katuri et al. (2017) and Shekhar and Singh (2020). The per cent efficacy of extracts was evaluated on the basis of reduction in tick count with in the observed area of 10 x 10 cm on the treated animals before treatment (‘0’ day) and on 24th hrs after treatment and then on 3rd, 7th, 14th and 28th day after treatment by using following formula (Sinha et al., 2010).

Clinical and hemato-biochemical evaluation
Clinical parameters namely temperature (°F), heart rate (per minute), respiratory rate (per minute), skin and hair coat condition, alertness, appetite, colour of conjunctival mucous membranes were recorded in all the animals on ‘0’ day (before treatment) and on 14th and 28th day after treatment. Hematological parameters viz., Hemoglobin (Hb) (g/dl), Packed Cell Volume (PCV) (%), Total Leukocyte Count (TLC) (x 10^3/cu.mm), Total Erythrocyte Count (TEC) (x 10^6/cu.mm), Differential Leucocyte Count (%), Mean Corpuscular Volume (fl), Mean Corpuscular Haemoglobin (pg), Mean Corpuscular Haemoglobin Concentration (g/dl) and Platelet Count (x 10^5/cu.mm) were estimated on fully automated cell counter ABAXIS make (Model VetScan H5S) on ‘0’ day (before treatment) and on 14th and 28th day after treatment. Biochemical parameters AST (IU/L), ALT (IU/L), ALP (IU/L), GGT (IU/L), Total Bilirubin (mg/dl), Direct Bilirubin (mg/dl), Serum Total Protein (gm/dl), Serum Total Albumin (gm/dl), Serum Total Globulin (gm/dl), BUN (mg/dl) and Serum Creatinine (mg/dl) were analysed on ‘0’ day (before treatment) and on 14th and 28th day after treatment by using readymade kits on biochemical auto-analyzer (Rapid Diagnostic Pvt. Ltd. STAR 21 Plus).

Evaluation of dermal reactions in cattle
After application of extracts, all the animals under study were also observed for visible dermal reactions at the site of application of extract, like staining, pruritus, erythematic lesion, skin irritation, swelling, reddening and other signs if any at different time intervals before and after application.

RESULTS AND DISCUSSION
The tick count in 10x10 cm areas on different regions of animal body mostly dewlap, neck, shoulder, chest, brisket, thighs and udder/scrotum was carried out on ‘0’ day (before treatment) and 24 hrs, 3rd, 7th, 14th and 28th day after treatment. The per cent efficacy of extracts was evaluated on the basis of reduction in tick count with in the observed area of 10 x 10 cm on the treated animals before treatment (‘0’ day) and on 24th hrs after treatment and then on 3rd, 7th, 14th and 28th day after treatment by using following formula (Sinha et al., 2010).

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<th>Table No. 2 Mean tick count and per cent efficacy at various treatment intervals</th>
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The same superscript in a row for particular treatment did not differ significantly. (NS: Non significant)

The figures in parenthesis indicates per cent efficacy.

The average pre-treatment tick count in T1 and T2 group was 55.80 ± 11.29 and 107.60 ± 28.12, respectively. The average tick count in group T1 and T2 was 49.90 ± 11.08, 35.40 ± 7.95, 24.00 ± 5.74, 17.90 ± 7.35, 11.70 ± 2.87 and 97.00 ± 27.85, 83.30 ± 29.75, 70.30 ± 30.51, 62.70 ± 32.97, 18.80 ± 5.05 on day 1, 3, 7, 14, and 28, respectively.
14 and 28, respectively (Fig. 1). The average tick count was progressively reduced after treatment at various intervals in both the groups. The statistical analysis revealed significant variation between different intervals in group T1 with significant reduction in tick count from 7th day (24.00 ± 5.74), 14th day (17.90 ± 7.35) and 28th day (11.70 ± 2.87) post treatment as compared to pre-treatment tick count (55.80 ± 11.29). In T2 group, the average tick count was found non-significant at various treatment intervals, however, it was markedly reduced on 28th day (18.80 ± 5.05) after treatment as compared to pre-treatment tick count (107.60 ± 28.12).

The per cent efficacy of extracts were evaluated on the basis of reduction in tick count with in the observed area at various treatment intervals are detailed in Table 2 and Fig. 2. Both the groups showed progressive increase in per cent efficacy at various intervals post treatment. However, per cent efficacy was higher in T2 group (82.53%) than T1 group (79.03%) on 28th day after treatment. Kumar et al. (2000) recorded maximum efficacy on 30th day after spraying of AV/EPP/14 herbal eotoparaciticidal compounds containing neem on cattle. Bagherwal (1999) found reduction in tick count on 16 to 20 days after second spray application of AV/EPP/14 neem containing herbal acaricide. Kaaya et al. (2007) reported significant reduction in the number of immature and adult ticks attaching on cattle for a period of 4 to 5 days when they applied 25% Neem oil on Zebu cattle. NRC (1992) also opined that increasing the number of consecutive sprays, enhances the activity of plant extracts under field condition as ultraviolet rays causes degradation of botanical extracts.

The reduction in tick count might be due to the most prominent phytoconstituent azadirachtin present in A. indica leaves extract. Azadirachtin is a tetranaotriterpenoid plant limonoid, which could have acted as an antifeedant, repellent and larvicidal in our study. The azadirachtin has been reported to act as a potent growth-disrupting agent having an antifeedant effect and causing a delay in the production of ecysone (Rembold et al., 1984 and Radcliffe et al., 1990). It acts as a disruptors of insect neuroendocrine functions by directly acting on corpora allata and prothoracic glands or indirectly by inhibiting the trophic actions of neurosecretory cells. These actions of azadirachtin was validated by Brotodjojo and Arbiwati (2016) in their study against insects using neem leaf powder and reviewed by Chaudhary et al. (2017).

The mean rectal temperature (°F) in T1 and T2 group was in the range of 100.00 ± 0.04 to 100.86 ± 0.20 and 101.02 ± 0.14 to 101.54 ± 0.11 respectively at different treatment intervals. The mean heart rate (beats per minute) in both the groups on day ‘0’ (before treatment) and at different intervals after treatment ranged between 67.30 ± 1.10 to 69.70 ± 1.22 and 65.80 ± 0.63 to 68.50 ± 1.06, respectively. The mean respiratory rate in T1 and T2 groups was ranged from 20.50 ± 0.48 to 21.10 ± 0.80 and 20.60 ± 0.31 to 20.90 ± 0.53, respectively at different intervals. The statistical analysis revealed non-significant variations in the rectal temperature, heart rate and respiratory rate at various treatment intervals in both the treatment groups and found within normal reference (Constable et al., 2017) ranges for the cattle. Milk yield in both the groups remained unaltered throughout study period and did not differ significantly in all the treatment groups at various intervals indicating no adverse effects of extracts on milk production.

The skin and hair coat of cattle was rough before application of extracts which might be due to tick infestation causing itching and rubbing of skin by bitting of ticks which leads to alopecia. The skin coat becomes smooth and shiny after treatment from 14th day with reduction in tick burden. These findings are similar with Kataviya et al. (2018), who demonstrated the acaricidal efficacy of novel indigenous herbal veterinary medication in the control of tick infestation against naturally affected cattle and found reduction in the pre-treatment tick count without any reactions at the application site and healthier skin conditions post treatment. Bhikane et al. (2018) also noticed significant reduction in clinical signs like itching, alopecia, redness, rubbing, pruritus and coarse skin coat with varying degrees of hair fall after treatment with polyherbal spray containing neem. They reported improved luster or shine on their hair coat on the 21st day post-treatment in cattle.

The mean haemoglobin (g/dl) in the range of 9.22 ± 0.39 to 10.04 ± 0.80 and 9.14 ± 0.25 to 9.47 ± 0.44 in T1 and T2 group, respectively at different intervals. The mean packed cell volume was in the range of 28.47 ± 1.65 to 28.87 ± 1.05 and 28.59 ± 0.80 to 30.06 ± 1.35, in T1 and T2 group, respectively at different intervals. The total erythrocyte count was in the range of 6.22 ± 0.43 to 6.37 ± 0.38 and 5.74 ± 0.31 to 6.44 ± 0.30 in T1 and T2 group, respectively at different intervals. The total leucocyte count was in the range of 9.16 ± 0.52 to 10.38 ± 0.97 and 7.13 ± 0.50 to 8.60 ± 0.90, in T1 and T2 group, respectively at different intervals. The platelet count was in the range of 2.94 ± 0.32 to 3.93 ± 0.47 and 2.97 ± 0.35 to 3.18 ± 0.55 in T1 and T2 group, respectively at different intervals. The mean haemoglobin showed increasing trend which might be due to reduction in tick count in all the treatment groups on 28th day post treatment as compared to 0th day. The statistical analysis revealed non-significant variation in Hb concentration, packed cell volume, total erythrocyte count, total leucocyte count and platelet count, between different intervals in all groups and remained within normal reference ranges for cattle (Constable et al., 2017). Das et al. (2015) determined the comparative efficacy of neem oil, tobacco and ivermectin against ectoparasites in Black Bengal goats and found increased values of Hb, PCV and TEC at 21 and 28 days of post treatment. However, Rajendran and Hafeez (2003) recorded reduction in Hb, PCV, TEC and TLC in tick infested animals before treatment as compared to after treatment.

The mean corpuscular volume (MCV) (fl) was in the range of 46.16 ± 1.93 to 49.83 ± 2.72 and 46.94 ± 1.54 to 50.49 ± 1.63, the mean corpuscular haemoglobin (MCH) (pg) was ranged between 14.68 ± 0.61 to 16.72 ± 0.86 and 14.80 ± 0.54 to 16.15 ± 0.60, the mean corpuscular haemoglobin concentration (MCHC) (g/dl) ranged from 31.91 ± 0.65 to 33.64 ± 0.37 and 31.53 ± 0.59 to 32.33 ± 0.70 in T1 and T2 group, respectively at different intervals. The statistical analysis of MCV, MCH and MCHC values revealed non-significant variation between different treatment intervals in all the groups and were within normal limits (Constable et al., 2017). Similar non-significant changes in MCV, MCH and MCHC were also reported by Rajendran and Hafeez (2003). Kumar et al. (2010) reported non-significant differences in MCV values between treatment and control animals. They also reported lower values of MCHC in infected animals than control animals because of tick infestation.

The neutrophil per cent was in the range of 29.20 ± 2.64 to 35.60 ± 4.88 and 27.00 ± 2.02 to 34.50 ± 1.73, in T1 and T2 group, respectively at different intervals. The lymphocyte per cent was ranged from 59.60 ± 5.39 to 66.40 ± 2.71 and 58.30 ± 1.37 to 62.00 ± 2.56 in T1 and T2 group, respectively at different intervals. The eosinophil per cent was in the range of 6.10 ± 3.37 to 4.60 ± 1.67 and 6.10 ± 0.82 to 3.90 ± 0.89 in T1 and T2 groups, respectively. The monocyte count was in the range of 0.20 ± 0.20 to 0.70 ± 0.37 and 2.80 ± 0.47 to 4.90 ± 0.77 in T1 and T2 groups, respectively. The eosinophil per cent was found higher on ‘0’ day (before treatment) in all treatment groups may be due to allergies caused by higher tick burden, however, it was found non-significant between different...
intervals in both the groups. The eosinophil per cent showed decreasing trend in both the groups on 28th day as compared to '0' day. The basophil per cent was zero in both the treatment groups at different intervals. Kumar et al. (2010) reported lower values of neutrophil per cent in infected animals than control animals because of tick infestation and reported non-significant differences in eosinophil and basophil per cent between treatment and control animals. Rajendran and Hafeez (2003) recorded significantly high eosinophil per cent in tick infested animals as compared to after treatment and non-significant changes in neutrophil, basophil, lymphocyte and monocyte count in treated and untreated animals. In the present investigation DLC study revealed non-significant variation in neutrophil, basophil, monocyte and lymphocyte per cent in both the treatment groups at different intervals and found within normal reference range (Constable et al., 2017).

The mean AST (IU/L) values was in the range of 65.60 ± 7.37 to 77.52 ± 7.13 and 50.69 ± 7.39 to 65.58 ± 6.60 in T1 and T2 group, respectively at different intervals. The mean ALT (IU/L) values was in the range of 21.96 ± 2.02 to 31.53 ± 4.82 and 20.90 ± 3.43 to 26.02 ± 5.31 in T1 and T2 group, respectively at different intervals. The mean ALP (IU/L) values was in the range of 61.49 ± 7.71 to 71.49 ± 15.39 and 50.07 ± 5.90 to 53.47 ± 6.47 in T1 and T2 group, respectively at different intervals. The mean GGT (IU/L) values was in the range of 14.83 ± 1.73 to 19.48 ± 1.54 and 16.24 ± 2.08 to 19.06 ± 1.55 in T1 and T2 group, respectively at different intervals. The statistical analysis revealed non-significant variation in AST, ALT, ALP and GGT values between different intervals in all groups which were within normal reference limits (Constable et al., 2017 and Brar et al., 2004) in cattle. Kumar et al. (2010) found considerably normal values of liver enzymes after treatment in tick infested animals.

The mean values of total bilirubin, direct and indirect bilirubin was in the normal range of cattle in T1 and T2 group at different intervals. The statistical analysis revealed non-significant variation in bilirubin values between different intervals in both the groups and found within normal reference limits (Constable et al., 2017 and Brar et al., 2004) in cattle. The mean total protein (gm/dl) values was in the range of 5.00 ± 0.20 to 5.06 ± 0.33 and 4.90 ± 0.36 to 5.58 ± 0.33 in T1 and T2 group, respectively at different intervals. The mean albumin values found in the range of 2.94 ± 0.11 to 3.03 ± 0.13 and 2.86 ± 0.14 to 3.00 ± 0.13 in T1 and T2 group, respectively at different intervals. The statistical analysis revealed non-significant variation in total protein and albumin values between different intervals in all groups and were within normal reference limits (Constable et al., 2017 and Brar et al., 2004) in cattle. On the contrary Rajendran and Hafeez (2003) recorded decrease in total protein and albumin levels in tick infested groups may be due to blood sucking by ticks. Kumar et al. (2010) reported higher values of proteins after treatment as compare to before treatment. These findings are similar with Bhikane et al. (2018) who studied the effect of acaricidal treatment with a polyherbal spray containing Azadirachta indica (1%) against tick infestation in cattle and revealed no significant changes in total protein, albumin and globulin levels before and after treatment.

The mean BUN values was in the range of 14.52 ± 0.86 to 16.00 ± 1.44 and 17.32 ± 1.71 to 18.57 ± 2.07 in T1 and T2 group, respectively at different intervals. The mean creatinine values were in the range of 2.19 ± 0.18 to 2.33 ± 0.15 and 2.16 ± 0.19 to 2.33 ± 0.13 in T1 and T2 group, respectively at different intervals. The statistical analysis revealed non-significant variation in BUN and creatinine values between different intervals in all groups and found within normal reference limits (Constable et al., 2017 and Brar et al., 2004). After application of extracts cattle were also evaluated for visible dermal reactions at the site of application of extract, like staining, pruritus, erythematic lesion, skin irritation, swelling, reddening and other signs at different time intervals before and after treatment. None of the animals from both the groups showed abnormal dermal reactions indicating extracts were non-toxic and safe for application on to the cattle. Similar findings also observed by Bhikane et al. (2018) who did not reported any adverse reactions like irritation, itching and salivation after treatment with polyherbal spray containing neem in cattle even after 21st day post treatment.

CONCLUSION

Both the groups showed progressive increase in per cent efficacy at various intervals post treatment. However, per cent efficacy was higher in T2 group (82.53%) than T1 group (79.03%) on 28th day after treatment, may be due to increase in the number of consecutive sprays which enhanced the activity of plant extracts under eld condition. The reduction in tick count might be due to the most prominent phytoconstituent azadirachitin present in A. indica leaves extract.

After application of 70% aqueous extracts of A. indica extract, none of the animals from both the groups showed abnormal dermal reactions and alterations in clinical, haematological and biochemical parameters at different time intervals after treatment. Hence, 70% aqueous extract of A. indica leaves found non-toxic and safe for application on to the cattle.

Declarations

ETHICS APPROVAL

The Departmental Research Ethics Committee from Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Postgraduate Institute of Veterinary and Animal Sciences, Akola-444104, India has approved this study.

COMPETING INTERESTS

Authors declare that there no competing interests

CONFLICTS OF INTEREST
There is no conflicts of interest.

**AUTHORS CONTRIBUTION**

Bhikane A. U. and Waghmare S. P. contributed to the study conception and design. Hajare S. W. helped in preparation of extracts. Ingole R. S., Kuralkar S. V. and Kolte S. W. analyzed the data. Hatzade R. I. executed study and written first draft of manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**DATA AVAILABILITY**

The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

**CONSENT TO PARTICIPATE**

Informed consent was obtained from all individual authors regarding submission of manuscript to Veterinary Research Communications.

**CONSENT TO PUBLISH**

Informed consent was obtained from all individual authors for publication of Figure 1 and 2.

**References**


Figure 1
Total tick count (mean) in treatment groups

Figure 2
Per cent efficacy of extracts