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Review Article *Stress Management*

Occurrence of Acaricidal Resistance on Ticks and Their Detection Measures

J. K. Chamuah*, Amenti and Lalchamliani

ICAR-NRC on Mithun, Medziphema, Nagaland (797 106), India

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Corresponding Author

J. K. Chamuah

e-mail: drjayantavet@gmail.com

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Abstract

Ectoparasites is one of the important cause for reduce growth and production of the animals. Among the ectoparasites, tick is the major causative agent for tick paralysis, tick injury, tick pyaemia along with transmission of different micro-organism to host animals. Acaricide resistance is a global phenomenon in all countries due to continuous and repeated use of acaricide in organized farms in years after year for long period of time. Predominance of resistance has been commonly observed in *R. microplus* in entire globes along with Indian subcontinent. However, occurrence of resistance against different hard tick, soft tick along with other ectoparasites has been documented in different form in different countries of the world. Acaricide resistance are initially present in very low proportion among ticks and would pass through next generation by reproduction of identical progeny. Resistance against acaricide acquired through target site modifications by mutation in the amino acid composition, increase in metabolic detoxification and excretion rate like esterase mediated point mutation is mainly responsible for occurrence of resistance in ticks and fly. Although use of Adult immersion test, Larval Packed test is the OIE recommended test for detection of acaricide resistance test. However molecular detection method is also quite convenient test for detection of acaricide resistance. In order to control this problem, change of medicated drugs, use of herbal drugs, and immunoprophylaxis along with management procedure may control this problem in today's context.

Keywords: Ectoparasite, tick, acaricide resistance, treatment

1. Introduction

Ticks are one of the major obligate ectoparasite causing harm to livestock in different form as mechanical injury, tick paralysis and transmitting different micro-organism to host animals. Due to suitable environmental condition prevailing in the region, the tick population is at high level and causing severe health problem in free ranging animals. The North eastern hilly region region provides ideal condition mainly because suitable climate in the form of temperature, humidity and high rainfall which are congenial for for perpetuation of life cycle of different ticks. The control of ticks is solely focused on repeated application of synthetic chemicals leading to development of acaricidal resistance. The problem is further accelerated by the fact that many tick populations have become resistant to one or other of the acaricide used to control ticks. The evaluation of resistance was recorded against synthetic pyrethroids (deltamethrin and cypermethrin) in *Rhipicephalus (Boophilus) microplus* ticks collected from nine districts of three agro-climatic zones of north-western

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Himalayan region of India (Gadara et al., 2019). However, reports of acaricidal resistance against chemical compounds very less. (Eiden et al. 2017). In this present article, occurrence of different ticks with application of chemical acaricide and acaricide resistance detection measure has been elaborately discussed in order to suitable measure against these emerging problems in different organized farms.

As per definition of World Health Organization Scientific Group (1965) is "the ability of a parasite strain to survive and to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject" (Abbas et al*.,* 2014). Resistance against acaricide acquired through target site modifications and increase in metabolic detoxification and excretion rate (Rosario-Cruz et al., 2009). In case of pyrethroids, target site modification is achieved by mutation in the amino acid composition which in turn confers resistance to the acaricide. Metabolic detoxification of acaricides involves multigene- families of enzymes such as glutathione-S-transferases, esterases and mixed function oxidases (cytochrome P450) which are thought to enhance detoxification by increased hydrolysis (Rosario-Cruz et al., 2009). These three enzymes are regulated by different molecular mechanism along with over expression of detoxifying enzyme-encoding genes DNA amplification. The over expression by up-regulation changes such as in cytochrome P450 genes cause point mutation in the GABA receptor and sodium channel target genes.

Resistance genes in tick populations are present in very low proportions initially before the particular chemicals were ever used. In ticks, genetic resistance allows resistance pass through by reproduction of resistant progeny individuals. Tick acaricide resistance is the ability of a strain of ticks to tolerate doses that would prove lethal to the majority of individuals in a normal population of the same species. Resistance may be conferred by a single gene or may be polygenic and may involve one or thought to be found in chromosomal DNA. Initially resistance genes are rare in a tick population but as selection by acaricides continue the proportion of resistance genes increases (Rodriguez-Vivas et al., 2011). Parasite genetic factors play a role in development of resistance and these include dominance of resistance alleles, number of genes involved, initial proportion of resistance genes, genetic diversity of the tick population and potential for genetic recombination (Georghiou & Taylor 1977). The amount of time required for resistance genesis also depends on the mode of inheritance of the resistant allele (dominant, co-dominant or recessive), the frequency of treatment with acaricide and the proportion of the presence of the population in refugia, which is not exposed to acaricides.

In esterase-mediated form of resistance, point mutation is observed within the active site of *Ach E* was also documented that two different amino acid substitutions within the same esterase gene confer resistance to organo phosphorus insecticide against sheep blow fly. SNP found within the *B. microplus* para type sodium channel gene is responsible for conferring resistance to pyrethroids.

2. Occurrence of Tick in Domestic Livestock

In India, almost all the livestock species suffer from tick infestations which results in tremendous production loss (Ghosh et al., 2007). Besides its effect on growth and production, tick infestations pose significant threat to the availability of good quality of hides and skin for leather industry which has been facing tremendous shortfall of good quality hides and skin to meet the demand. A 20-30% reduction in the cost of leather due to tick bite marks alone has been estimated (Biswas, 2003).

Due to free ranging nature of mithun, the problem of tick infestation is acute. The one host tick, *Rhipicephalus (Boophilus) microplus* is the most commonly recorded tick species in mithun inhabited in Nagaland (Chamuah, 2005). However, few cases of *Haemaphysalis daivasi* and *H. neumani* infestation in mithun have also been reported (Miranpuri and Naithani, 1978). According to a study conducted on 176 numbers of mithun, it was observed that 58.52% Mithuns under the study were infested with *R. (B.) microplus* (Chamuah et al., 2013). Intensity of infestation is higher on older animals and the highest prevalence was recorded in the summer season (Rajkhowa et al., 2003). The *Amblyomma testudinarium* species has recently been recorded in Assam, Nagaland, Mizoram and Manipur. This species was earlier reported states like Assam (Miranpuri et al., 1976; Lahkar,1991), Meghalaya (Hoogstraal and Rack ,1967), Arunachal Pradesh (Dhanda and Rao ,1964; De and Gupta, 1978) and Sikkim (Saravanan et al., 2008) an various animals like mithun, tiger, wild boar, barking deer and elephant.

3. Application of Chemical Acaricides

Globally, application of four classes of chemical acaricides (organophosphates, pyrethroids, foramidine and macrocyclic compounds) are the mainstay of tick control program. The cases of *R. (B.) microplus* developing resistance to organophosphates and pyrethroids (Miller et al., 2005 & 2007; Davey et al., 2006) are well documented. Recently, resistant monitoring tools were standardized and validated (Jyoti et al., 2013; Singh et al., 2010). The status of resistance in ticks infesting animals of NEH region has not been studied.

Acaricidal plant extracts may be a potential substitute for synthetic acaricides currently used tick. The essential oils *of Azadirachta indica* and of *Ocimum suave* have shown acaricidal and repellent properties against the larvae of tick species (Abdel-Shafy and Zayed, 2002). The acaricidal property of the essential oils of *Melaleuca alterifolia* Cheel (tea tree oil) against nymphs of *Ixodes ricinus* has been demonstrated (Iori et al., 2005). *Dermanyssus gallinae* could be controlled by the use of Azadirachtin impregnated cardboard traps (Lundh et al., 2005). Extracts from various African plants have been shown to possess strong acaricidal properties (Kayaa Godwin, 2000). Promising results were reported of when some plant extracts were used against *R. (B.) microplus* (Morais-Urano et al., 2012). Essential oils from leaves and flowers of *Ageratum houstonianum* and essential oil of *Origanum onites* and *O. minutiflorum* were reported to possess acaricidal activity against *R. (B.) annulatus* and *R. turanicus (*Tedonkeng et al., 2005; Cetin et al., 2009). Pure metabolites from root extracts of *Pimenta alliacea* showed insecticidal properties (Lyndon et al., 1997) while the stem extracts of the same plant contain benzyltrisulfide (BTS) and benzyldisulfide (BDS) metabolites having potent acaricidal activity (Rosado-Aguilar et al., 2010). Cadina-4, 10 (15)-dien-3 one isolated from the leaves and stems of *Hyptis verticillata,* collected from Jamaica, disrupted the oviposition and hatching of *R.* (*B.) microplus* eggs (Porter et al., 1995). Moreover, Martin et al. (2001*)* isolated 5 sesquiterpenoids, 4 flavonoids and p-coumarate ethyl from plants of *Polygonum pimctalnm* and also reported acaricidal activity. But none of the compounds were tested against ticks resistant to chemical acaricides.

In India, Khudrathulla and Jagannath (2000) studied the effect of a methanolic extract of *Styloxanthes scabra* on ixodid ticks. The leaves of tobacco (*N. tabacum*) were found effective against *R. haemaphysaloides (*Choudhary et al., 2004). Methanolic extracts of neem (*A. indica*) leaves and barks were tested for the acaricidal effects (Pathak and Mathur, 2004). The acaricidal property of neem seed extracts has been evaluated against *B. microplus* adults in vitro and in vivo*.* The prepared extract was found effective against 70.5% of the challenged ticks (Srivastava et al., 2008). Ethanolic extract of *C. fistula* leaves completely inhibited hatching of tick eggs, while ethanolic extract of *C. alata* produced highest mortality (45.8%) and inhibition of fecundity (10.9%) (Sunil et al., 2013; Ravindran et al., 2012). In a comprehensive study, 95% ethanolic extract of *R. communis* was tested in vitro against cattle ticks. The extract significantly affected the mortality rate of ticks with an additional effect on reproductive physiology by inhibiting oviposition (Ghosh et al., 2013). Recently, Ghosh and Ravindran (2014) reviewed the status of phytoacaricides research and identified few plant species for the development of eco-friendly formulation for tick control.

As in the case of other parts of the country, the tick control is focused on application of chemicals. However, because of limited use of chemical pesticides possibly ticks have not developed resistance to the commonly used chemicals and it is high time that botanicals are introduced after appropriate study of the tick situation there. Recently, through a systematic study the anti-tick potentiality of three solvent guided plant extracts were identified and protocols for studying the resistance pattern in ticks and processing of plant materials were developed and validated (Ghosh et al., 2013,). All the three identified potential plant species are grown in the NEH regions. The advantage is that there are a number of undisturbed natural habitats where these species are found abundantly. Therefore, it is expected that a greater range of natural variability in the plant species will be available to study the anti-tick activity and this will help in conservation of the best plant accession for development of eco-friendly sustainable region specific tick control strategy.

4. *In Vitro* Diagnostic Methods for Detecting Acaricidal Resistance

4.1. Larval packet test (LPT)

It is mainly used by the commonwealth scientific and industrial Research organization (CSIRO) and in Latin America and Africa. FAO has promoted this test as Acaricide Resistance testing kit. In this test, tick larvae are exposed to different acaricides and their subsequent mortality is estimated. As per FAO modification, larval packet test is commonly used for 12-14 days old larvae. As the standard test protocol, a dilution series of chemical drug is made to concentration gradient resulting 0-100% larval mortality. Whatman filter paper is dipped for two minutes in 2ml of each concentration. After saturation, it is dried in incubator at 37°C. Chemically treated Whatman filter paper id folded into equilateral triangular packets and sealed on the sides with adhesive paper forming an open ended packet. Approximately 100 larvae is put in the packet and placed in desiccators kept in BOD maintained at 28°C and 75-80% RH. After 24 hours in BOD, the number of dead and live larvae is counted. The ability of the larvae to walk on the surface of the filter paper is used as the criteria to determine their mortality.

Mortality = Total no. of tick larvae in packet –live tick larvae.

% Percentage of mortality= No. of dead larvae/Total No. of larvae × 100

The dose response data is analyzed by Probit analysis. The 50% lethal concentration (LC) of acaricides against R. (B.) microplus is determined by applying regression equation analysis to the probit-transformed data of mortality. Using LC 50 values of reference lines, resistance factor (RF) was worked out by the formula given by Castro-Janer et al. (2010):

Resistance factor (RF) = LC50 Value of field ticks/LC50 Value of susceptible ticks

On the basis of RF, the resistance status in the field population of *R. (B.) microplus* was classified as susceptible (RF < 1.4), level I resistance (1.5 < RF < 10.0), level II resistance (10.1 < RF < 25.0), level III resistance (26 < RF < 40), and level IV resistance (RF > 41).

4.2. The adult immersion test (AIT):

Adult immersion test is widely used to determine the relative effectiveness of engorged female ticks against new acaricides and it was originally described by Drummond et al. (1973), which was later on modified by Agricultural Research Service, United States Department of Agriculture. In this method, engorged female tick is immersed in 10ml of different concentration of tested chemicals and control ticks in normal plain water. Immersed engorged female ticks are placed on petridishes over whatman filter paper at room temperature for 24 hours. After 24 hours, ticks are transferred to glass vials covered with muslin cloth and kept in desiccators having 75-80% RH inside BOD incubator at 28°C for oviposition and observed for minimum 15 days. The laid egg mass is observed under same condition of BOD at 28 °C for 15-30 days and the percentage of adult tick's mortality and the weight of the eggs laid by treated ticks is recorded in comparison with control immersed in water. The index of egg laying and percentage inhibition of fecundity is calculated using the following formulae, respectively.

Reproductive Index (RI) = Weight of eggs laid (mg)/ weight of adult females (mg)

Percentage of inhibition of oviposition (IO %) = RI (Control group)-RI (Treated groups) x100/RI (Control groups).

4.3. Molecular detection of Acaricidal Resistance

Molecular characterization is a new approach to study the resistance to acareicides in populations of *Rhipicephalus microplus* (Acari: Ixodidae) larvae. The DNA from the larvae is extracted according to standard kit protocols followed by quantification and quality evaluation by nanodrop. To determine the resistance and susceptibility of the larvae to the organophosphate chemical group, the primer pair GS138B 5'-GCATCGACCTCTCGTCCAAC-3' and GS139R 5'-GTCGGCATACTTGTCTTCGATG-3' is used, as described by Hernandez et al. (2002). The PCR reaction is performed as per the following protocols of prior denaturing at 95 °C for 5 min, followed by 10 cycles at 95 °C for 1 min, 65 °C for 1 min (with decrease of 1°C per cycle) and 72 °C for 1 min, followed by 30 cycles at 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 7 min. The PCR product was digested with the restriction enzyme EcoRI at 37 °C for 3 h and submitted to electrophoresis in 1.8% agarose gel, stained with 0.001% ethidium bromide and gel will be viewed under gel documentation system (Faza et al., 2013). Each sample is genotyped for the presence of the T2134A substitution in the S6 trans-membrane segment of domain III of the para-type sodium channel to detect the single nucleotide polymorphisms (SNP) (resistance allele R) in the sodium channel gene as described by He et al. (1999) using an allele specific PCR as reported by Guerrero (2001). The PCR is carried out in two separate reactions to detect both the pyrethroid susceptible and resistant alleles with the help of primers FG221 (5'–TTATCTTGGCTCCTTCT–3') and FG227 (5'–TTGTTCATTGAAATTGTCGA–3') for susceptible allele and FG222 (5'–TTATCTTCGGCTCCTTCA–3') and FG227 (5'–TTGTTCATTGAAATTGTCGA–3') to amplify the resistant alleles. PCR is performed as per the protocol at denaturing at 95 °C for 5 min, followed by 10 cycles with denaturing at 95 °C for 1 min, annealing at 65 °C for 1 min (with a decrease of 1 °C per cycle) and extension at 72 °C for 1 min, followed by 30 cycles with denaturing at 95 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min and final extension at 72 °C for 7 mins. Genotypes were identified on the presence or absence of a 64 bp diagnostic amplicons. The 64 bp fragment amplified with the FG221/FG424 primer pair characterizes susceptibility (allele A) to pyrethroids and the 64 bp fragment amplified with the FG222/FG424 primer pair characterizes resistance (allele B). The presence of the two fragments indicates moderate resistance of the adult ticks against this chemical compound.

5. Conclusion

In acaricide resistance, even though in north-eastern region not reported scientifically, however quite possibly existed in domestic livestock from this part of country. Therefore, along with scientific community farmers of this region should be aware with condition and preventive measure should be taken in the farm of alteration of chemicals, use of herbal drugs, and genetic selection of individual breeds of domestic livestock with proper integrated tick control measure in near future.

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