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**Citation for published version:**

Matika, O, Foster, S, Githaka, N, Mwendia, C, Brown, H, Watson, K, Djikeng, A & Birkett, M 2022, 'In search of a new tool for phenotyping tick resistance in cattle', World Congress on Genetics Applied Livestock Production, Rotterdam, Netherlands, 3/07/22 - 8/07/22. <[https://www.wageningenacademic.com/pb-assets/wagen/WCGALP2022/27\\_004.pdf](https://www.wageningenacademic.com/pb-assets/wagen/WCGALP2022/27_004.pdf)>

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## In search of a new tool for phenotyping tick resistance in cattle

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### Abstract

Ticks and tick borne diseases cause significant loss in livestock production with about 80% world's cattle at risk. The cost of chemical control is high and there is an ever increasing tick resistance to chemicals. Genetic selection as alternative long term control strategy is constrained by laborious phenotyping using tick counts or scores. This study explores the use of host volatile semiochemicals that may be attractants or repellents to ticks as a novel phenotype with potential to be used as a proxy in selection programmes. About a 100 young cattle composed of *Bos indicus* and *Bos Taurus* were artificially infested with 2,500 *R. decoloratus* larvae with daily female ticks (4.5mm) counts taken from day 20 post infection. Volatile compounds we sampled before and after tick infestation. We identified three pre-infestation volatile compounds that were associated with tick resistance (p-value < 0.01) and one post infestation (p-value < 0.05) using 6 day repeated measure analysis. The high correlation coefficients (r=0.66) between repeated records with all volatile compounds support the potential predictive value for volatile compounds in selective breeding programmes for tick resistance in cattle.

### Introduction

Ticks and tick borne diseases cause significant loss of production with at risk about 80% of the world's cattle. Economic losses from ticks and tick-borne diseases have been estimated to range from US\$20-30 billion annually (Lew-Tabor and Rodriguez Valle, 2016). Host resistance to ticks has been reported as moderately to highly heritable (Burrow and Henshall, 2014) which translates into a long term, low-cost intervention and requires no extra labour or resources (Frisch, 1999). Although host resistance to ticks can be considered as one of the most economical tick control strategies, improvements in host resistance have been largely neglected (Frisch, 1999). The main reason is that the gold standard tick phenotyping methodology involves tick counts which are laborious, time-consuming and require skilled animal technicians as well as expensive infrastructure to constrain animals. A simpler, cost-effective method of identifying individual animal variation in resistance to ticks (phenotyping) under research, commercial and smallholder production systems is urgently needed to enable improvements in host resistance to ticks. In the past, knowledge of different tick pheromones, allomones and kairomones has been used to develop novel tick control products by incorporating tick pheromones and small amounts of pesticide to attract and kill ticks on their hosts or in vegetation (Latha, 2012). Ticks like other haematophagous arthropods use host olfactory and visual cues known as volatile semiochemicals, which generally regarded as attractants, but also can be repellents in some cases (Logan and Birkett, 2007). In order to use

the semiochemicals as proxies for tick resistance, we need to quantify within animal variation of different semiochemicals and how they relate to tick loads. In this study, we investigate within animal variation in tick burdens between *Bos Taurus* and *Bos indicus* cattle using artificial tick infestation and quantifying differences in volatile semiochemical production. Evidence for a correlated response between volatile semiochemical and tick loads could underpin a development of a new low-cost and rapid tool for phenotyping cattle for resistance to ticks, with potential use for selection and animal breeding in both commercial and smallholder cattle production systems.

## **Materials and Methods**

### **Animals, ticks and tick infestation.**

The study animals composed of 24 males and 28 females East African Zebu cattle (*Bos Indicus*) aged 12 to 18 months purchased from local farmers in Western Kenya were transported to the study site at ILRI, Nairobi, Kenya and male *Bos Taurus* (45 Holstein-Friesian and 9 Ayrshire) steers from ILRI farm. Cattle were artificial infested with 2,500 *Rhipicephalus* (*Boophilus*) *Decoloratus* (*R. decoloratus*) larvae, with daily counts of female ticks (4.5mm) conducted over a 6-day period from day 20 post infestation.

### **Volatile compounds sampling and gas chromatography (GC) analysis.**

Each animal was held in a crush and volatile organic compounds (VOCs) were collected using air entrainment using the anterior groin of an animal by a hand-held air sampling pump. The funnel was held gently against the skin to ensure a seal around the funnel perimeter. Collections were carried out for 5 minutes. Two samples were collected per animal, i.e. prior to, and 21-23 days after, tick infestation.

VOCs were analysed by high resolution gas chromatography-flame ionization detector (GC-FID) analysis. Nine VOCs (isoamyl acetate, (*E*)-2-heptenal, benzaldehyde, 6-methyl-5-hepten-2-one, hexanal, decane, (*RS*)-limonene, undecane and tridecane) were selected as candidates for investigation based on previous reports as non-host semiochemicals for ticks.

### **Statistical Analysis.**

Volatile organic compound data were analyzed separately as nine pre- infection values (BI), nine post infection (AI) values and nine differences (diff) between the pre and post infection values resulting in 27 volatile compound traits. The tick counts were treated as six daily counts modelled as repeated measures or each daily count as separate trait and total or average. The distribution of raw tick counts and volatile compounds were mostly skewed; hence they were transformed to approximate normality using  $\log_{10}(x + 1)$  with a constant added to allow for the zero counts in our data. Data were subsequently analysed in SAS software (SAS, 2012) for repeated measure analysis modelling the six days tick count effects in the model. The final model fitted for the repeat measure analyses included effects of day, group, body weight and volatile compounds. The fixed effect of “group” was created by combining the effects of sex (Male or Female) and breed (Zebu or Taurus) to give a group effect for male Zebu, female Zebu and male Taurus animals.

Single trait analysis fitted individual daily tick counts, tick totals or averages analysed using GLM (SAS, 2020) using general linear models but removing the effects of day in the models.

The models explored both breed and sex as fixed effects or group, plus the covariates already mentioned above.

Trait ~ breed +sex (or group) + body weight + volatile compounds

Where trait was individual daily log transformed tick counts, log transformed tick totals or log transformed tick averages.

The correlation between transformed tick counts and volatile organic compounds was explored using Pearson correlation coefficients using SAS (2012).

## Results

There was wide variation between pre- infection values (BI, CV% ranging from 18.4 to 59.4) and post infection (AI, CV% ranging from 8.2 to 65.2) across sexes and breeds. The CV% was generally higher for BI in female Zebu than Males, except for volatiles BI-(*E*)-2-heptenal/benzaldehyde) and BI-(*E*)-2-heptenal. The CV% of log transformed tick counts ranged from 31.1 to 84.0.

The repeat measure analysis before infestation identified three volatile compounds (BI-(*E*)-2-heptenal, BI-6-methyl-5-hepten-2-one and BI-hexyl acetate) that were significantly (p-value <0.05) associated with low tick counts suggesting that they could be used as predictive indicators of tick resistance. All fixed and covariate effects of day, group, body weight and volatile compounds significantly (p-value <0.05) accounted for other variations in the model. Results for after infection tick counts for repeat analysis identified a single volatile compound (AItrans-2-heptenal/benzaldehyde) that was significant (p value <0.05) on volatile compounds.

There were three volatile compound differences (Diff(AI-BI): (*E*)-2-heptenal/benzaldehyde, 6-methyl-5-hepten-2-one, hexyl acetate)) that were significantly (p value<0.05) associated with tick counts whilst two of the compounds (6-methyl-5-hepten-2-one and hexyl acetate) were previously identified as significantly associated with tick counts in the BI results.

Five single day tick count and volatile compound combinations were identified in the GLM analysis as significant (p<0.05) where isoamyl acetate (TickD25 by AI-isoamyl acetate) and limonene (TickD25 by AI- limonene) had not been previously observed in the repeat measure analysis. Two other volatile compounds ((*E*)-2-heptenal/benzaldehyde and 6-methyl-5-hepten-2-one) were observed in the previous analyses with p-values ranging from <0.015 to 0.045. These volatile compounds represent potential proxies for use in predicting tick resistance without counting ticks on the animals. The volatile compound (lnBI6-methyl-5-hepten-2-one) was significantly (p-value <0.05) associated with tick counts during the first 3 days of sampling and also for tick count totals and tick average.

The high correlation coefficients, r=0.66 and r=0.73 respectively for models including repeated records or mean tick counts with all volatile compounds support the potential predictive value for volatile compounds in selective breeding programmes for tick resistance in cattle.

## Discussion

The within breed natural differential attractiveness of individual animals to disease-transmitting cattle flies was found to be partly due to differences in volatile semiochemicals emitted by the host (Birkett et al., 2004). Other studies (Jensen et al., 2004) reported a genetic basis to host resistance to cattle flies. High correlation between volatile

semiochemical production and tick resistance in cattle could underpin the development of a new low cost tool for phenotyping cattle for selection and breeding for tick resistance in livestock breeding programmes.

The work to develop new anti-tick vaccines are ongoing (Guerrero et al., 2014, Lew-Tabor & Rodriguez Valle, 2016), but new vaccines are highly unlikely to confer total protection against ticks. It has been postulated (Frisch, 1999), that the best method of reducing the impacts of these stressors to improve productivity and animal welfare is to breed cattle that are well-adapted to the different types of stress, and work in concert with other management interventions.

We identified volatile compounds with some naturally found in naïve animals, if this is can be replicated in other studies, it would allow sampling of animals irrespective of the tick infestation status. Results for post infestation compound can be taken to show host responses to an ongoing tick challenge. In sub-Saharan Africa, ticks and tick borne diseases are a major constraint, example of livestock tick-borne diseases reported in Kenya include theileriosis, babesiosis, anaplasmosis and ehrlichiosis, which reflect the diversity of the transmitting tick vectors (Mwamuye et al., 2017).

This search for potential new volatile-based resistance phenotype has the potential to simultaneously improve cattle resistance to both ticks and biting flies. The new phenotype would enhance predictive power across breeds and countries to enhance existing efforts already being undertaken in this regards (Cardoso et al. 2021). Our current method will need further refinement to satisfy the requirements of a simple, cost-effective phenotype available for use in large cattle herds as a replacement for the laborious tick counts.

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