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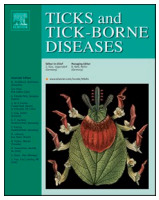

Original article

Investigating volatile semiochemical production from Bos taurus and Bos indicus as a novel phenotype for breeding host resistance to ixodid ticks

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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 chemical acaricides. Genetic selection as alternative long-term control strategy is constrained by laborious phenotyping using tick counts or scores. This study explored the use of host volatile semiochemicals that may be attractants or repellents to ticks as a phenotype for new tick resistance, with potential to be used as a proxy in selection programmes. Approximately 100 young cattle composed of *Bos indicus* and *Bos taurus* were artificially infested with 2,500 African blue tick, *Rhipicephalus decoloratus* larvae, with daily female tick (4.5 mm) counts taken from day 20 post-infestation. Volatile organic compounds were sampled from cattle before and after tick infestation by dynamic headspace collection, analysed by high-resolution gas chromatography (GC) and subjected to multivariate statistical analysis. Using 6-day repeated measure analysis, three pre-infestation GC peaks (BI938 - unknown, BI966 - 6-methyl-5-hepten-2-one and BI995 – hexyl acetate) and one post-infestation GC peak (AI933 – benzaldehyde / (*E*)-2-heptenal) were associated with tick resistance ($P < 0.01$ and $P < 0.05$ respectively). 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.

1. Introduction

About 80% of the world's cattle are at risk of ticks and tick-borne diseases, both of which cause significant production losses. Economic losses from ticks and tick-borne diseases were estimated in 1996 to range from US$13.9 – 18.7 billion per annum (de Castro, 1997), with more recent 2015 estimates ranging from US$20 to 30 billion annually (Lew-Tabor and Valle, 2016). Approximately 80 ixodid tick species have been identified in Kenya (Walker et al., 2003). The major tick-borne diseases of livestock in Kenya include theileriosis, babesiosis, anaplasmosis and ehrlichiosis, reflecting the diversity of the transmitting tick vectors (Mwamuye et al., 2017). Although there is lack of accurate up-to-date data on the economic losses due to ticks and tick-borne diseases in Kenya, Kenya spent approximately US$ 10 million to control ticks and tick-borne diseases in 1987 (Young et al., 1988). Current losses, which are 30 years later, are considerably higher. Furthermore, the spread of the highly invasive cattle tick *Rhipicephalus microplus* across Africa over the past two decades has been predicted to exacerbate the problem (Kanduma et al., 2020; Githaka et al., 2021). White et al. (2003), assessed that Australian beef industry were vulnerable to impacts of ticks under a climate change scenario and predicted significant expansions in potential geographical impacts, with increased abundance of tick populations and reductions in cattle productivity. Tick control measures including grazing practices (Pfeffer et al., 2018; Nicaretta et al., 2020) and use of chemicals have limitations such as acaricide resistance (Foil et al., 2004; George et al., 2004; Guerrero et al., 2012;

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Rodriguez-Vivas et al., 2012; Abbas et al., 2014; Bandara and Karunaratne, 2017; Chitombo et al., 2021; Dzemo et al., 2022; Yawa et al., 2022), contamination of animal products such as meat and milk, inadequate rotational grazing areas etc. However, breeding for host resistance to tick and tick-borne diseases offers a complementary and sustainable control method compared to existing methods. In a review of tick control methods, Frisch (1999) suggested that cattle host resistance was the single most important factor affecting the economics of tick control. The published heritability estimates for host tick resistance in literature range from low (0.00) to high (0.89) (Fraga et al., 2003; Budeli et al., 2009; Mapholi et al., 2016; Biegelmeyer et al., 2017, 2017) depending on breeds, environments, parasite challenge and methods of assessment used. Investing in genetic improvement represents a cost effective intervention, long term and permanent solution to tick burdens (Frisch, 1999; Regitano and Prayaga, 2010). However, Frisch (1999) observed that this most economical-tick control strategy was largely neglected. The primary reason for such neglect is the difficulty and cost of identifying individual genetic variation in resistance to ticks. This constraint applies in research herds as well as in commercial and smallholder farms and particularly in low to middle income countries (LMICs). To date, researchers have used single or repeated counts of the number of engorging ticks (i.e., ticks between 4.5 and 8 mm in diameter for *Boophilus* sub-genus on one side of each animal following artificial or natural infestation (Wharton and Utech, 1970) to identify individual animal variation in tick resistance. Tick counts are time-consuming and require skilled animal technicians as well as expensive infrastructure to constrain animals simultaneously. 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. Burrow et al. (2019), in a review, proposed the use of three other possible phenotypes (haemolytic analysis; measures of skin hypersensitivity reactions; simplified artificial tick infestations) that can be developed to determine their practical feasibility for consistently, cost-effectively and reliably measuring cattle tick resistance in large herds both in commercial and smallholder systems in tropical and subtropical areas. Other novel methods such the use of immune competence assays (Robbertse et al., 2017) are to be fully explored as future proxies for tick resistance phenotypes.

Ixodid ticks and other haematophagous arthropods use olfactory and visual cues in host location, with olfactory cues emanating from the bodies and breath of their hosts predominant in the process. These behaviour-modifying olfactory cues, known as volatile semiochemicals, are generally regarded as attractants, though in some cases they can also be repellents (Logan and Birkett, 2007). Over the past 10–20 years, 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). However, existence of within animal variation of semiochemicals associated with host tick resistance could form the basis for assessing host tick resistance in cattle. Previous research involving Holstein-Friesian cattle, *Bos taurus*, and disease-transmitting cattle flies in the Netherlands and Denmark (Birkett et al., 2004), demonstrated that natural differential attractiveness of individual animals within the breed was partly due to differences in volatile semiochemicals emitted by the host. Furthermore, there was a genetic basis to host resistance to cattle flies. Birkett et al. (2004) showed that variation in cattle fly loads were determined by differences in individual host volatile semiochemical production. Other work has also explored volatile compounds variation and tick counts for domestic dog breeds (Borges et al., 2015; Zeringota et al., 2021), and horses (Ferreira et al., 2019). Furthermore, the ability to link within animal variation in ectoparasite load with volatile semiochemical production offers the prospect of delivering a novel method to identify tick resistant animals. In order to use the semiochemicals as proxies for tick resistance, we need to quantify within animal variation of different semiochemicals and how these relate to tick loads. In this study, we investigate within animal variation in the African blue tick, *Rhipicephalus decoloratus* (Acari: Ixodidae) tick burdens between *B. 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.

2. Materials and methods

2.1. Study animals

All animal procedures in this study were authorised by the International Livestock Research Institute (ILRI) Institutional Animal Care and Use Committee under approval no. IACUC2019-28. Young East African Zebu cattle (*B. indicus*) aged 1–1.5 years old, of which 24 males and 28 females were purchased from local farmers in the western Kenyan county of Busia and transported to ILRI, Nairobi, Kenya. Cattle were vaccinated for foot and mouth disease (FMD) and kept in quarantine for 3 weeks prior to date of first sampling. During this period, the animals were kept off acaracide treatments and provided with water and hay grass *ad lib* and their health monitored daily. Any animal *in extremis* during the experiment was removed from the study. All male *B. taurus* (45 Holstein-Friesian and 9 Ayrshire) approximately 1–1.5 years old steers from the ILRI farm in Nairobi were held in separate pens from the *B. indicus* for the duration of the trial. All animals had body weight (kg), height at withers (cm), measure of circumference or heart girth (cm), dentition and blood taken pre-infestation. Prior to enrolment, study animals were screened by serology for common tick-borne pathogens and only negative animals were selected. This was in part to meet regulatory requirements for animal movement across the country and avoid animals with active infections. After the study, the animals were returned to the ILRI farm whereas three taurine animals were removed from the study due to poor condition.

2.2. Ticks

For artificial infestation and evaluation, larvae were sourced from an existing laboratory colony of the African blue tick, *R. decoloratus* that is maintained and propagated at the ILRI Tick Unit according to standard tick rearing procedures. Briefly, this entails applying tick larvae on 6–9-month-old calves and allowed to feed until repletion and detachment at day 21–22. Engorged female ticks detaching from the animals were recovered, washed, weighed and transferred to petri dishes for oviposition. Ticks were incubated at 28 °C and 85% relative humidity. Following hatching, tick larvae were used within 3 months to ensure viability and successful infestation of animals.

2.3. Tick infestation

Rhipicephalus decoloratus larvae (2500) were applied on each animal on the back and the withers using a brush at 09.00 h and allowed to move freely over the whole animal. Tick infestation and counting was conducted in cattle animals using crushes to restrain the animals. Daily tick counts of standard engorged female ticks (4.5 mm) were conducted over a 6-day period, starting at day 20 post tick-infestation. The tick counting method was based on Wharton and Utech (1970).

2.4. Dynamic headspace collection of volatile organic compounds from cattle

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 entrainment of air

containing VOCs was achieved by taking samples from the anterior groin of an animal using a hand-held Personal Air Sampling Pump ESCORT ELF® (Zefon International Inc.) supplying an air flow (flow-rate 1 L/min) which was drawn through a TENAX TA trap (50 mg polymer, 60/80 mesh, Supleco, Bellefonte, PA) that was secured inside a Bohlender™ PTFE funnel using PTFE tubing and an O ring (ID 11 mm, OD 16 mm). The funnel was held gently against the skin to ensure a seal around the funnel perimeter. The trap was connected to the pump by Tygon tubing (90 cm length x 0.9 cm OD). TENAX TA traps were conditioned before use by washing with distilled diethyl ether (4 mL) and heating at 132 °C under a stream of nitrogen. Prior to use, traps were sealed in glass ampoules under an inert atmosphere of N_2, and after collections were complete, re-sealed in ampoules and stored at −20 °C until required for chemical analysis. Collections were carried out for 5 min. Two samples were collected per animal, i.e. prior to, and 21–23 days after, tick infestation. Background collections of VOCs were made prior to animals being moved into the crush.

2.5. Gas chromatography (GC) analysis

VOCs were analysed by high-resolution gas chromatography-flame ionization detector (GC-FID) analysis. The VOCs trapped on TENAX TA were analysed on a GC instrument (Agilent Technologies, 6890 N, Stockport, UK) equipped with a flame ionization detector (FID), a Programmable Temperature Vaporization (PTV) Unit (ATAS, Cambridge, UK) and a 50 $m \times 0.32$ mm i.d. non-polar HP-1 column (0.52 µm film thickness). TENAX tubes were inserted into the PTV unit and heated balistically (30->250 °C at a rate of 16 °C/s). The oven temperature was maintained at 30 °C for 1 min and programmed at 5 °C/min to 150 °C, where it was held for 0.1 min, then at 10 °C /min to 250 °C and held for 27 min. The carrier gas used was hydrogen. For each GC-FID analysis, the GC retention times (in minutes) were converted to GC retention indices (RI) using an alkane mix (Millar and Haynes, 1998). Peak areas (in pA) for GC peaks with RI values closely matching (less than or equal to 1 unit difference), previously reported as non-host tick semiochemicals, i.e. isoamyl acetate (861), (*E*)−2-heptenal (933), benzaldehyde, 6-methyl-5-hepten-2-one (966), hexanal acetate (995), decane (1000), (*RS*)-limonene (1026), undecane (1100) and tridecane (1300), and an additional compound at GC peak (RI 938) identified from preliminary analysis of GC-FID data were selected as candidates for investigation. All nine collected VOCs were later used in subsequent statistical analysis (See Supplementary Table S1).

2.6. Statistical analysis

Traits – Volatile organic compound (VOC) data were analysed 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 (See Supplementary Table S1). The tick counts were treated as six daily counts modelled as repeated measures or each daily count as separate trait and total or average.

Descriptive Statistics – Preliminary data analysis was conducted using PROC UNIVARIATE, SAS software (SAS, 2012) to investigate if the assumption normality was valid for tick counts (across 6 days from day 20 post infection) and the volatile compounds (pre and post infection) traits. 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.

Repeat measure analysis – Data were subsequently analysed fitting PROC MIXED (SAS, 2012) for repeated measure analysis modelling the six days tick count effects in the model. Fixed effects for breed (Zebu or Taurus), sex (male or female) or group (male Taurus, male Zebu and female Zebu) were accounted for in the models explored. In addition, effects of body weight (kg), height at withers (cm), heart girth (cm) and volatile compounds were also investigated as covariates in the analyses. First order interactions were also fitted for the fixed effects and covariates. 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. The final model to determine environmental factors influencing tick count and volatile compounds were by fitting the following fixed effects models:

$$Y_{ijkm} = \mu + D_i + G_j + (B*V)_{lm} + b(W_k) + c(V_m) + e_{ijklm} \quad (1)$$

$$Y_{ijkm} = \mu + D_i + G_j + (G*V)_{jm} + b\,(W_k) + c(V_m) + e_{ijkm} \quad (2)$$

where: Y_{ijlkm} is repeated daily log transformed tick counts all modelled as a single trait; µ is the overall mean; B_i is the effect of the i^{th} Day (i=days 20…25); G_j is the effect of the j^{th} Group (j= male Zebu, female Zebu or male Taurus); $(B*V)_{lm}$ is the interaction effect of the l^{th} Breed and m^{th} volatile compound; $(G*V)_{jm}$ is the interaction effect of the j^{th} Group and m^{th} volatile compound; b is the regression coefficient of body weight on tick count; W_k is the effect of the animal body weight; c is the partial regression coefficient of volatile compounds on tick count; V_m is the effect of the volatile compounds with e_{ijklm} and e_{ijkm} are the random residual errors depending on the model fitted.

Single trait analysis – Individual daily tick counts, tick totals or averages were analysed fitting PROC GLM (SAS, 2012) which is a least squares method using general linear models but removing the effects of day in the models 1 and 2 described above. In this model, we fitted both breed and sex as fixed effects or group, plus the covariates already mentioned above (see model below).

Trait = breed +sex (or group) + body weight + volatile compounds (3)

Where trait was individual daily log transformed tick counts, log transformed tick totals or log transformed tick averages. The animal cohort groups before the start of the trial were fitted in the models only for BI data analysis.

Correlation Analysis – The correlation between transformed tick counts and volatile organic compounds was explored using Pearson correlation coefficients on phenotypic and residual values after accounting for the model terms using PROC CORR in SAS (2012) fitting all variables.

3. Results

We observed eight previously identified volatile tick semiochemicals from GC-FID analysis followed by comparison of GC retention indices with authentic standards i.e. isoamyl acetate (862), benzaldehyde/(*E*)−2-heptenal (931), 6-methyl-5-hepten-2-one (967), hexyl acetate (995), decane (1000), (*RS*)-limonene (1023), undecane (1100) and tridecane (1300) in collected VOC samples from both *B. taurus* and *B. indicus* cattle (see supplementary Table S1). In addition, we identified a novel GC peak with a RI of 938 also appeared to segregate between samples and so was included in statistical analysis. The log transformation was the most appropriate method approximating normality for both tick counts and volatile compounds and was later used in all the subsequent analyses.

Descriptive statistics (mean, standard deviation, minimum, maximum and coefficient of variation (CV)) for log transformed volatile compounds and tick counts are given respectively in Supplementary Tables S2, S3 and S4. There was considerable variation (CV%) between pre- infection values (BI, CV% ranging from 18.4 to 59.4, Table S2) and post infection (AI, CV% ranging from 8.2 to 65.2, Table S2) across sexes and breeds (data not shown). The CV% was generally higher for BI in female *B. indicus* than males, except for volatiles BI-(*E*)−2-heptenal/benzaldehyde) and BI-(*E*)−2-heptenal and was lowest in male *B. taurus*. Mean daily log tick counts peaked on day 22 to 24 and the CV% was highest on day 20 at 84.0 and lowest at day 22 at 31.1 (Table S3). Highest tick counts were recorded on day 22 in both male and female

B. indicus, and male *B. taurus*, however higher counts were observed in *B. taurus* (Table S4).

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 <0.05) accounted for other variations in the model (Table 1). However, the interaction between breed or group with volatile compounds were not significant (P >0.05) when fitted (data not shown). Results for post-infestation tick counts for repeat analysis identified a single volatile compound (AI: trans-2-heptenal/benzaldehyde (AI933)) that was significant (P <0.05) in the model (Table 2) with no interactions of breed or group on volatile compounds (P>0.05, Data not shown). 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 <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 (Table 3a). The only volatile compound that was significant (P <0.05) when we fitted a VOC*Breed interaction model was Diff (AI-BI) hexyl acetate (995) (Table 3b).

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 (Table 4). These volatile compounds represent potential proxies for use in predicting tick resistance without counting ticks on the animals. The volatile compound (lnBI: 6-methyl-5-hepten-2-one) was significantly (P <0.05) associated with tick counts during the first 3 days of sampling and also for tick count totals and tick average. The full results of volatile compounds with potential for further investigation are given in Table 4. The effects of breed and sex (or group) were significant (P<0.05) in most of the traits considered.

The results of one of the highly significant predictors of tick counts across day 20 to 25 for volatile compound (BI: 6-methyl-5-hepten-2-one (BI966) are given in Table 5 together with those for tick totals and tick averages. The volatile compound BI: 6-methyl-5-hepten-2-one was significantly (P <0.05) associated with tick counts during the first 3 days of sampling. Moreover, tick count totals or average were both associated (P<0.05) with this compound (Table 5).

There were high correlation coefficients, $r = 0.66$ (P<0.0001) and $r = 0.73$, (P<0.0001) respectively for models including repeated records or mean tick counts with all volatile compounds. These results support the potential predictive value for volatile compounds in selective breeding programmes for ixodid tick resistance in cattle.

4. Discussion

The current study found eight previously identified volatile tick semiochemicals from differentially preferred domestic dog breeds (Borges et al., 2015; Zeringota et al., 2021) and horses/donkeys (Ferreira et al., 2019), and discovered one novel VOC segregating within both *B. taurus* and *B. indicus* cattle. We observed differences between sexes and breeds. However, more importantly, we observed that all nine VOCs were segregating in both breeds and sexes before artificial tick infestation. This may be because animals had previously encountered the ticks prior to the trial and their immune system was not naïve to tick challenge or that the animals have a natural genetic predisposition to exude these VOCs. If the latter is the case, then this will allow VOCs sampling in herds naturally grazed or in extensive rearing systems where prior tick infestation is uncertain. 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). In another study, 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. Previous work has shown that volatile tick semiochemicals emitted by less-preferred animal hosts have the potential to be used as repellents for reducing tick populations (Birkett et al., 2004; Borges et al., 2015; Ferreira et al., 2019; Zeringota et al., 2021). However, deployment of such repellents requires the development of slow-release formulations which, particularly for repellent blends comprising more than one component, can be technically challenging, with a risk of sub-optimal activity ensuing. Despite the availability of novel dispenser technologies that could facilitate commercial development of repellents, a more sustainable approach is to enhance the production of repellents in animal hosts, by exploring their biosynthesis, and therefore, the genetic factors that are responsible for repellent production.

Although previous attempts have been made to define the variation in Kenyan cattle resistance to ticks (de Castro et al., 1991), these studies were insufficiently rigorous to generate reliable phenotype data for tick resistance. Thus, a new study, undertaken here, was required to create a repository of phenotypes to underpin collection and analysis of volatile semiochemical production, to test the hypothesis that there is variation in tick burden between *B. taurus and B. indicus* cattle under artificial challenge and that the observed variation is associated with quantifiable differences in volatile semiochemical production. *Rhipicephalus decoloratus*, the tick species used in the current study follows the one-host life cycle and remains attached on the host until fully replete females drop on the ground to restart the life cycle through egg laying. It is therefore plausible that volatile semiochemicals are continuously secreted following tick challenge including during larval and nymphal stages that occurs before the 21-day post-infestation sampling point. Whether different instars induce secretion of different compounds or at different intensities was not investigated in the current study (adults obviously

Table 1
P-values of repeated *Rhipicephalus decoloratus* (Acari: Ixodidae) tick measure analysis of 6 day (from day 20 post infection) tick counts fitted with before infestation (BI) volatile compounds fitted as covariates.

	BI Volatile Compounds fitted as covariates								
Factors	861	933	938	966	995	1000	1026	1100	1300
Group	0.0031	0.0025	0.0025	0.0030	0.0026	0.0031	0.0482	0.0609	0.0037
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wt	0.0466	0.0496	0.0253	0.0216	0.0309	0.0482	0.0196	0.0480	0.0432
Volatile (BI)	NS	NS	**0.0135**	**0.0002**	**0.0118**	NS	0.0609	NS	NS

The volatile compounds are denoted by their GC peaks as follows: isoamyl acetate (861), (E)−2-heptenal (933), benzaldehyde, 6-methyl-5-hepten-2-one (966), hexanal acetate (995), decane (1000), (RS)-limonene (1026), undecane (1100) and tridecane (1300), and a novel compound at GC peak (RI 938); Wt~ body weight, group effect ~ male Zebu, female Zebu and male Taurus.

Table 2
P-values from repeated *Rhipicephalus decoloratus* (Acari: Ixodidae) tick measure analysis of 6 day (from day 20 post infection) tick counts fitted with after infestation (AI) volatile compounds fitted as covariates.

	AI Volatile Compounds fitted as covariates								
Factors	861	933	938	966	995	1000	1026	1100	1300
Group	0.0001	<0.0001	0.0001	0.026	<0.0001	0.0001	0.0001	0.0001	0.0002
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wt	0.0263	0.0645	0.026	0.0201	0.015	0.0231	0.0223	0.0234	0.0236
Volatile (AI)	NS	**0.038**	NS	NS	NS	NS	NS	NS	NS

The volatile compounds are denoted by their GC peaks as follows: isoamyl acetate (861), (E)−2-heptenal (933), benzaldehyde, 6-methyl-5-hepten-2-one (966), hexanal acetate (995), decane (1000), (RS)-limonene (1026), undecane (1100) and tridecane (1300), and a novel compound at GC peak (RI 938); Wt ~ body weight, group effect ~ male Zebu, female Zebu and male Taurus.

Table 3a
P-values from repeated *Rhipicephalus decoloratus* (Acari: Ixodidae) tick measure analysis of 6 day (from day 20 post infection) tick counts fitted with difference between before (BI) and after infestation (AI) volatile compounds (Diff(AI-BI)) fitted as covariates.

	Diff(AI-BI) volatile compounds fitted as covariates								
Factors	861	933	938	966	995	1000	1026	1100	1300
Group	0.0001	<0.0001	0.0002	0.0002	<0.0001	0.0001	0.0002	0.0001	0.0001
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wt	0.0246	0.0625	0.0179	0.0104	0.01	0.0233	0.0201	0.0283	0.0266
Diff(AI-BI)	NS	0.065	**0.087**	**0.0009**	**0.003**	NS	NS	NS	NS

The volatile compounds are denoted by their GC peaks as follows: isoamyl acetate (861), (E)−2-heptenal (933), benzaldehyde, 6-methyl-5-hepten-2-one (966), hexanal acetate (995), decane (1000), (RS)-limonene (1026), undecane (1100) and tridecane (1300), and a novel compound at GC peak (RI 938); Wt ~ body weight, group effect ~ male Zebu, female Zebu and male Taurus.

Table 3b
P-values from repeated *Rhipicephalus decoloratus* (Acari: Ixodidae) tick measure analysis of 6 day (from day 20 post infection) tick counts fitted with difference between before (BI) and after infestation (AI) volatile compounds (Diff(AI-BI)) fitted as covariates and interaction between volatile compounds and breed.

	Diff(AI-BI) volatile compounds fitted as covariates								
Factors	861	933	938	966	995	1000	1026	1100	1300
Group	0.0001	<0.0001	0.0003	0.0002	<0.0001	<0.0001	0.0006	0.0001	0.0001
Day	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wt	0.0318	0.0521	0.0184	0.0078	0.0045	0.0290	0.0302	0.0290	0.0270
Diff(AI-BI)	NS	**0.0179**	NS	**0.0235**	**0.0111**	NS	NS	NS	NS
Diff(AI-BI)*breed	NS	NS	NS	NS	**0.0500**	NS	NS	NS	NS

The volatile compounds are denoted by their GC peaks as follows: isoamyl acetate (861), (E)−2-heptenal (933), benzaldehyde, 6-methyl-5-hepten-2-one (966), hexanal acetate (995), decane (1000), (RS)-limonene (1026), undecane (1100) and tridecane (1300), and a novel compound at GC peak (RI 938); Wt ~ body weight, group effect ~ male Zebu, female Zebu and male Taurus.

Table 4
P-values from a GLM single trait analysis of daily *Rhipicephalus decoloratus* (Acari: Ixodidae) tick counts with different after infestation (AI) volatile compounds (/) fitted as covariate.

	Log transformed tick count with different volatile compounds as covariates					
Factor/ Trait	lnTickD24/ lnAI861	lnTickD25/ lnAI861	lnTickD20/ lnAI933	lnTickD25/ lnAI933	lnTickD20/ lnAI966	lnTickD25/ lnAI1026
Breed	<0.0001	<0.0001	0.109	<0.0001	0.090	<0.0001
sex	0.012	0.058	0.959	0.028	NS	0.029
Wt	0.017	0.143	0.724	0.317	NS	0.084
volatile	**0.047**	**0.015**	**0.062**	**0.015**	**0.018**	**0.018**
R2	0.46	0.44	0.14	0.44	0.16	0.44

LnTickD20…LnTickD25~ daily log transformed tick counts from day 20 to day 25; 861, 933, 966 and 1026 represent the following volatile compounds: isoamyl acetate, (E)−2-heptenal, benzaldehyde, 6-methyl-5-hepten-2-one and (RS)-limonene, respectively; Wt ~ body weight, breed ~ Zebu or Taurus.

imbibe more blood and inject larger amounts of salivary and saliva components that modulate host immune responses including those targeting innate immunity and possibly associated with host resistance and emission of volatile semiochemicals). Ticks of the subgenus *Boophilus* build up to large numbers in susceptible hosts potentially leading to disproportional large tick challenge compared to other tick genera. We also investigated the before infestation VOCs as this state may depict naïve animal VOC secretion. The build-up of ixodid ticks on hosts is also partially linked to the nutritional status of the animal, which itself may be related to the nature of the VOCs that the animals are secreting e.g. starving animals might be metabolising differently and displaying varying metabolites (Tolleson et al., 2010, 2012).

Beef and dairy cattle production in tropical and sub-tropical environments encounters numerous types of stressors ranging from ecto-parasites (ticks, flies, other haematophagous arthropods), endoparasites (gastro-intestinal helminths, flukes, protozoa), seasonally poor nutrition, heat and water stress, high humidity to diseases that are often caused by a diverse set of pathogens (bacteria, fungi, viruses). Under the

Table 5
Example of p-values from a GLM single trait analysis for log transformed *Rhipicephalus decoloratus* (Acari: Ixodidae) tick counts for volatile compound (benzaldehyde, 6-methyl-5-hepten-2-one (lnBI966)) fitted in the model as a covariate.

	Different Log transformed tick count with LnBI966 volatile compound as covariates							
Trait	lnTickD20	lnTickD21	lnTickD22	lnTickD23	lnTickD24	lnTickD25	lnticktot	lntickave
Breed	0.010	0.031	0.004	0.021	0.000	0.000	0.000	0.000
sex	0.790	0.021	0.013	0.021	0.027	NS	0.016	0.018
cohortbi	0.001	0.050	0.809	0.977	NS	NS	0.576	0.553
wt	0.756	0.014	0.005	0.004	0.010	0.096	0.008	0.006
lnBI966	**0.06**	**0.012**	**0.033**	**0.098**	0.105	0.104	**0.027**	**0.033**

LnTickD20…LnTickD25~ daily log transformed tick counts from day 20 to day 25; lnticktot, lntickave ~ log transformed tick totals and average, respectively; Wt ~Body weight, breed ~ Zebu or Taurus.

extensive production systems common in the tropics, it is generally not possible to control the stressors through management strategies alone, and even if intervention strategies were feasible, the treatments often cause their own problems. Acaricides and anti-tick vaccines do not offer a permanent solution to tick control. Acaricide treatments used to control ticks are losing efficacy due to the rapid development of resistance (Abbas et al., 2014; Dzemo et al., 2022; Githaka et al., 2022; Yawa et al., 2022), and acaricide use generates concern about residues in meat and milk products (Uilenberg, 1996; Beys-da-Silva et al., 2020), in addition to environmental effect on non-target organisms.

In a recent review, Burrow et al. (2019) examined the factors affecting tick resistance of cattle, the biological mechanisms of host tick resistance and possible alternative novel phenotype(s) for tick resistance. Any development and validation of new cost-effective phenotype (s) for tick resistance of cattle will have a significant role in generating proxies for use in genomic selection with the potential to improve host tick resistance (Cardoso et al., 2021). These proxies can be incorporated into breeding objectives to simultaneously improve cattle productive attributes and tick resistance. This study was conducted with the aim of identifying novel VOC phenotypes that have a potential to be proxy measures of host tick resistance that could be incorporated in breeding programmes for simultaneous improvement in cattle resistance to both ticks and biting flies. Since the current measurements of volatile chemistry do not satisfy the requirements of a simple, cost-effective phenotype for use in commercial cattle herds, we considered the inclusion of potentially simpler measures to enable indirect genetic selection for volatile-based resistance to ticks.

The work to develop new anti-tick vaccines are ongoing (Guerrero et al., 2014; Lew-Tabor and 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 and resilient 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 add to current tools available for tick resistance phenotyping but still needs 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. However, one tick species was used in a controlled artificial infestation environment on station, which means that the results need to be validated in other natural infestation environments where animals encounter multiple tick species and genera. Moreover, dedicated animal herds reared under tick-free environments are needed to minimise pre-study tick exposure. Therefore, the current study should be considered as a starting point for larger studies to characterise the estimates of heritability and genetic correlation with other production traits. This will mean investigating the relationship of the VOCs identified in this study with other tick species in outdoor extensive rearing systems that will resemble the target farming systems.

6. Conclusion

We identified nine VOCs, including one novel VOC, segregating within B. taurus and B. indicus cattle in Kenya. Our results indicated that some of these VOCs had potential to be predictive for host tick resistance.

Ethics approval

All animal procedures in this study were authorised by ILRI's Institutional Animal Care and Use Committee under approval no. IACUC2019-28.

CRediT authorship contribution statement

Oswald Matika: Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing. **Sarah Foster:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Naftaly Githaka:** Conceptualization, Funding acquisition, Writing – review & editing. **Gad Owido:** Conceptualization, Investigation, Writing – review & editing. **Collins Ngetich:** Conceptualization, Investigation, Writing – review & editing. **Charles Mwendia:** Conceptualization, Funding acquisition, Writing – review & editing. **Helen Brown:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **John Caulfield:** Conceptualization, Investigation, Writing – review & editing. **Kellie Watson:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Appolinaire Djikeng:** Conceptualization, Funding acquisition, Writing – review & editing. **Michael Birkett:** Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102200.

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