

The distribution and variation of Ixodid ticks in Matabeleland region Zimbabwe

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SUMMARY

Ticks have been known to cause severe economic losses due to cattle deaths, low milk production, poor beef quality and poor hide quality. To inform control strategies, it is necessary to carry out tick surveys to determine tick species present in Matabeleland as well as determine their distribution in the region. A total of 119 cattle were sampled from seven sites in Matabeleland communal and commercial areas, that is, Mangwe, Grills farm, Umguz, Beitbridge, Insiza, Gwanda and Nkayi. Phenotypic identification was done by microscopy and total DNA extraction was performed using the tick legs, which was then followed by Random Amplified Polymorphic DNA (RAPD) analysis to determine genetic variation and DNA polymorphism within the tick population. Of the cattle sampled, 268 ticks were collected and 11 tick species were identified, that is, *Amblyomma hebraeum* 54%, *Hyalomma truncatum* 11%, *Rhipicephalus evertsi evertsi* 12%, *Rhipicephalus appendiculatus* 7.4%, *Rhipicephalus (Boophilus) decoloratus* 6%, *Hyalomma rufipes* 3.7%, *Rhipicephalus zambeziensis* 1.7%, *Rhipicephalus sanguineus* 1.1%, *Rhipicephalus simus* 1.1%, *Rhipicephalus lunulatus* 0.7% and *Amblyomma variegatum* 0.7%. The occurrence of *Amblyomma variegatum* in this part of the country was a unique finding as the tick is known to be a northern tick and is a rare tick in the southern part of the country. Of the 11 tick species collected, only four tick species were used in the genetic characterization, that is, *Hyalomma truncatum*, *Hyalomma rufipes*, *Amblyomma hebraeum* and *Rhipicephalus lunulatus*. Primers OPY-3 and OPX-14 revealed genetic variation amongst the four species showing that RAPDs can be used for determining genetic diversity amongst tick population. This study forms a baseline in further studies on genetic characterization of ticks in Zimbabwe.

Key words: Ixodid ticks, distribution, Matabeleland, Zimbabwe, *Rhipicephalus*, *Amblyomma*, *Hyalomma*

INTRODUCTION

Ticks have been documented to transmit diseases that can result in significant losses of livestock (Koney, 2004).

Accordingly they remain a threat at large particularly for an Agro based economy such as that of Zimbabwe. A reduction in milk production can also occur in dairy cows which in turn will affect dairy industries and contributes to more economic problems. Ticks can also cause physical damage to body parts of ruminants (Ndhlovu *et al.*, 2009). Consequently cattle hides, which are a raw material in the tanning industry, can be damaged due to tick infestation and this can in turn grossly affect income acquired in industry. In Zimbabwe, the main method of tick control has been the use of acaricides, that is, pyrethroids, chlorinated hydrocarbon, arsenicals and of late amitraz.

However tick infestations still persist. Considering that Ixodid ticks have been documented as transmitters of diseases of economic importance in livestock, knowledge of tick distribution and variation is relevant to understand risks of infection transmission and disease occurrence and thus influence tick control measures. Sixteen tick species have been documented to occur

in Zimbabwe by Walker *et al.*, (2014). Quite occasionally also exotic (foreign such as Botswana) livestock have been brought into the country and may have carried in ticks not commonly found in the country. Such ticks may cause untold damage on local stock (Horak *et al.*, 2001) if unchecked.

There is also a need for continuous update on the presence and distribution of ticks and take note of new tick species in Zimbabwe as climate changes and land reforms are put in place (Sungirai *et al.*, 2015).

As structures which are usually essential for identification may become damaged during removal of ticks from their hosts, these difficulties may be avoided by using keys based upon molecular genetic markers. These techniques are becoming widely used to identify arthropods including ticks (El-Fiky *et al.*, 2003).

Thus the study was aimed at describing inter-population variation among ticks found in Matabeleland region of Zimbabwe with the aid of random primers so as to improve our taxonomic understanding of the species.

MATERIALS AND METHODS

Study area and Sampling

The study was approved by the National University of Science and Technology, Department of Applied Biochemistry and Biology's ethics committee. Tick samples were collected from cattle that originated from seven sites in the two provinces (Matabeleland South and Matabeleland North) in the Matabeleland region. The animals were brought to slaughter at two abattoirs in the regional capital city, Bulawayo, that is, Grills abattoir and Cold

Storage Company (Bulawayo division) in 2015. The abattoirs were selected on the basis that they are the authorized commercial abattoirs in the region. Animals at such stations have the history of the original province and owner of the animal documented. Random selection of the beef farming areas originating the animals sampled was done by identifying the farming areas that brought in animals first on the day of collection, and in sequential order. Names of the sites of origin of the animals were documented, both longitude

and latitude coordinates of the site and the dates of collection were noted (Figure 1). Samples were collected in the following sites around the Matabeleland region; Grills farm 20° 9' 12" S, 28° 58' 00" E, Umguza 20° 12' 15" S, 28° 42' 53" E, Mangwe 20° 42' 00" S, 28° 04' 00" E, Insiza 19° 47' 00" S, 29° 12' 00" E, Beitbridge 22° 13' 00" S, 30° 00' 00" E, Gwanda 20° 56' 42" S, 29° 01' 30" E and Nkayi 19° 00' 00" S, 28° 54' 00" E.

Two farming areas brought in cattle from Matabeleland North and five from Matabeleland South during the sample collection period. Information supplied by the farmers indicates consistent and fair use of acaricides by commercial farmers where cattle were sprayed with amitraz once every week in the summer.

Communal farmers, with cattle from Umguza, Grills farm and Beitbridge indicated slightly less use of acaricides where cattle were dipped in amitraz once every fortnight in summer. Information on breeds and age of animals was not given. Tick collections were performed on 119 adult cattle sampled randomly during summer, in the first two weeks of the month of December; 87 from Matabeleland and 32 from Botswana. In the first week cattle from

Matabeleland South (Grills, Gwanda, Beitbridge, Mangwe and Insiza) were collected, in sequential order of sites of origin.

In the second week cattle from Matabeleland North (Umguza and Nkayi) and Botswana were collected in sequential order of sites of origin. Cattle that were transported from Botswana during this period were all sampled. These animals were transported in heavy vehicles from the farming areas of origin to the two abattoirs for slaughter. The study used a simple randomized block design.

Tick collections were performed on all the cattle brought in from the randomly selected farming areas in Matabeleland region. A full body tick count was made on the hides of slaughtered cattle from the respective sample area using steel forceps. The hides presented the predilection attachment sites which were: the base of tail, perianal region, perineum, legs, udder, scrotum and belly.

Adult tick specimens were collected to allow morphological for easier identification due to their increased size. Animal slaughter and tick collection was done under a hygienic and monitored environment to prevent cross-infestation.

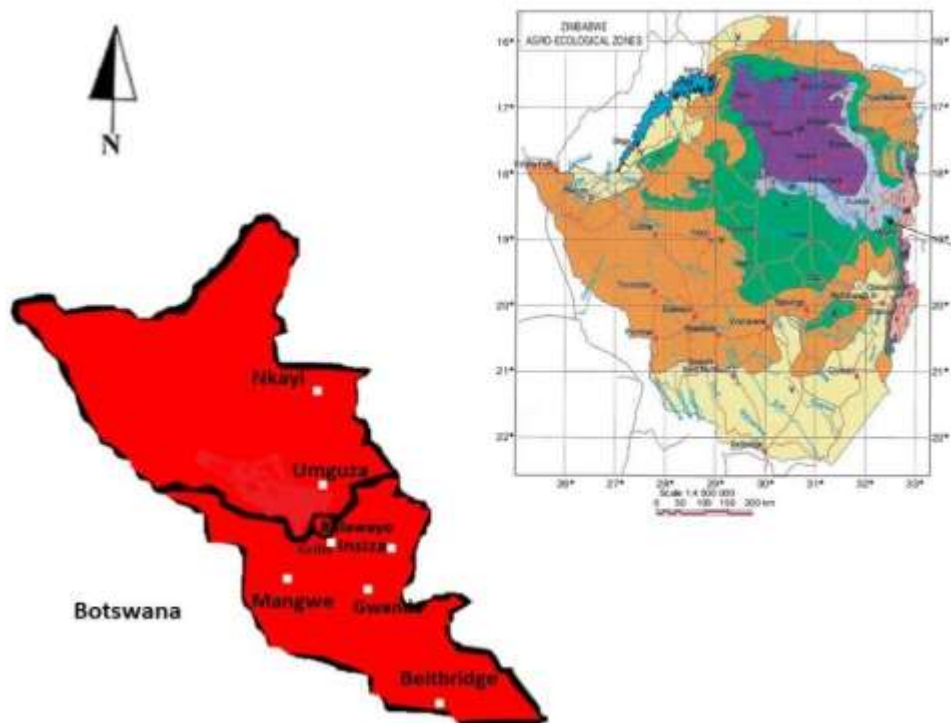


Figure 1: Map showing the locations of farming arrears where the sample Cattle originated from in Matabeleland Zimbabwe (Rukuni and Eicher (1994) in Chikumba *et al.*, 2006)

Phenotypic identification

Collected ticks were transported in plastic containers, grouped on phenotypic characterization using a dissecting light (stereo) microscope at the Department of Applied Biochemistry and Biology (Parasitology) at the National University of Science and Technology, Zimbabwe. The phenotypic identification of the ticks was done using identification keys as provided by Walker *et al.* (2014) for the *Rhipicephalus*, *Amblyomma* and *Hyalomma*. Ticks were then bled and stored in 70% ethanol for further analysis.

DNA extraction

DNA extraction was performed using the legs of ticks as there are minimal chances of non-tick DNA in the legs compared to the abdomen. Extraction was done as per laboratory protocol available (Halos *et al.*, 2004). Ticks were mechanically crushed

using a razor blade and white tile and lysed in 200µl lysis buffer (NaCl 0.1M, Tris-HCl 0.21M, pH8 EDTA 0.05M, SDS 0.5%) in eppendoff tubes. The sample was vortexed gently (3-5 seconds). Enzymatic digestion was by addition of 3µl of proteinase K with a concentration of 20µg/ml. Incubation was performed for 16 hours (56°C) while gently mixing at every 10 minutes.

DNA extraction was then carried out using 5M NaCl extraction instead of phenol-chloroform extraction. Twenty µl of 5M NaCl was added to the sample in lysis buffer and the sample was kept frozen during the extraction.

The sample was then centrifuged at 10 000g for 10 minutes and the supernatant eluted into fresh eppendoff tubes. The supernatant was kept in ice for 10 minutes and centrifuged again at 10 000g. The supernatant was then transferred into fresh

tubes. DNA was precipitated with absolute ethanol (two and half volumes) by incubation at -70°C for 1 hour in a freezer. Precipitation was completed by centrifuging at 10 000g for 10 minutes. Two DNA washing steps were done in 70% ethanol and DNA was re-suspended in 25µl of nuclease free water and stored at - 20°C. Electrophoresis, at 100V, 300mA and 30W, on 1% agarose (1g agarose in 100ml 1X TPE buffer) in 1X TPE for 30 minutes.

DNA was stained with 1µl of 6× loading dye (10mM Tris- HCl (pH7.6), 10mM EDTA, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60mM EDTA) for every 5µl of sample. Viewing of gels was enabled under UV light.

RAPD Polymerase Chain Reaction

Population polymorphisms were determined using random primers in Random amplified polymorphic DNA (RAPD) analysis. Selected primers were used to amplify the genomic DNA from 10 samples. A total reaction volume of 20µl of 2µl of 10X

Dream Taq Green buffer, 2µl of 10mM dNTPs mixture, 1µl of the specific primer, 2.875µl of MgCl₂, 0.165µl of Dream Taq polymerase. DNA was amplified using Gene Amp PCR systems 9700 with 40 cycles for 30 seconds of denaturation at 95°C, 45 seconds of annealing at 40°C and 1.5 minutes of primer extension at 72°C, with the initial denaturation at 95°C for 2 minutes and a final extension time of 15 minutes at 72°C.

Electrophoresis of PCR products was done on 1.5% agarose (1.5g agarose/100ml TBE buffer in X1 TBE buffer).

Primer screening

Three primers obtained from Eurofine® were screened in the study, that is, OPX-14, OPY-03 and OPO-05. The primers were used to compare among four taxa; *Hyalomma truncatum*, *Hyalomma rufipes*, *Amblyomma hebraeum* and *Rhipicephalus lunulatus*. OPY-03 was the primer of choice as it amplified all the samples.

RESULTS

Phenotypic identification

Ticks were identified phenotypically using morphological characteristics. The identified species includes *Amblyomma hebraeum*, *Amblyomma variegatum*, *Hyalomma truncatum*, *Hyalomma rufipes*, *Rhipicephalus evertsi evertsi*, *Rhipicephalus appendiculatus*, *Rhipicephalus (B) decoloratus*, *Rhipicephalus simus*, *Rhipicephalus zambeziensis*, *Rhipicephalus sanguineus* and *Rhipicephalus lunulatus*.

Of the 119 cattle sampled (87 from Matabeleland and 32 from Botswana), 61 were infested with ticks (Table 1). Thirty two cattle from Botswana were sampled and out of those only 11(34%) were infested

with ticks. Lower proportion (52%) of cattle were tick infested compared with cattle from communal farms in which the proportion was 63%. Commercial farms recorded approximately one and half times less cattle than in communal farms.

Of the ticks collected *A. hebraeum* contributed highest tick count in both the communal (61%) and the commercial (45%) sectors (Table 3). The back and neck accounted for the highest percentage (30 %) of the ticks collected *in situ*, followed by the upper perineum (26 %) with the tail switch (19 %) accounting for the lowest percentage.

The lower perineum accounted for 24% of tick infestation. *A. hebraeum* dominated the

highest prevalence on all the predilection sites. *R. evertsi evertsi* was significantly high on the upper perineum (35%) compared to other species. *R. appendiculatus* had a high count in the lower perineum (25%). *H. truncatum* had its highest prevalence is the tail switch (33%) and back (6%).

Irrespective of the tick species, the back and neck and the upper perineum recorded significantly higher mean tick counts compared to the other sites. *A. variegatum* occurred only in the back and lower perineum.

Table 1: Number of cattle infested with ticks and the prevalence of tick infestation according to farming area/sector

Farming area/sector	Number of cattle sampled	Number of cattle infested	Proportion (%)
Matabeleland South			
Communal: Gwanda	8	5	63
Insiza	14	10	71
Mangwe	2	1	50
Commercial: Beitbridge	7	3	43
Grills farm	18	10	56
Matabeleland North			
Communal : Nkayi	11	7	64
Commercial: Umguza	27	14	51
Total	87	50	57
Botswana	32	11	34

Association between tick infestation and udder and teat damage

The percentage of tick infested animals with udder damage was higher than those with tick infestation without udder damage. The percentage of tick infested animals with teat infestation was also higher than those without teat damage. Also observed is that teat damage was lower than udder damage in all sampled animals. Of all the ticks that were collected, male ticks account for 59% (159) and females 41% (109). The percentage of male ticks was significantly higher than female ticks $\chi^2(P<0.01)$. The highest number of male ticks was contributed by *A. hebraeum* (103) (65%).

Molecular characterization

DNA was extracted from fresh ticks, and 70% ethanol preserved ticks. Analysis of

the extraction of DNA, from the legs only, indicated that extraction from fresh samples yielded the highest success rate of DNA extracted (100%). Samples preserved in 70% ethanol had the least success rate of DNA extraction (30 %).

RAPD-PCR managed to amplify the samples used in the molecular analysis, that is, *Hyalomma truncatum*, *Hyalomma rufipes*, *Amblyomma hebraeum* and *Rhipicephalus lunulatus*.

Three primers amplified tick samples during primer screening. OPY-03 was the primer of choice as it amplified all samples and showed polymorphic bands among the four tick taxa. Amplification success was defined as the production of a visible PCR amplification bands. OPY-03 revealed that the four taxa possess more shared than distinguishing genetic characters.

Table 2: Total tick counts according to tick species identified by microscopy (Matabeleland region)

Tick species	Tick count	Proportion (%)
<i>A. hebraeum</i>	145	54
<i>A. variegatum</i> **	2	0.7
<i>H. truncatum</i>	30	11
<i>H. rufipes</i>	10	3.7
<i>R. evertsi evertsi</i>	33	12
<i>R.(B) decoloratus</i>	16	6
<i>R. appendiculatus</i>	20	7.4
<i>R. sanguineus</i>	3	1.1
<i>R. simus</i>	3	1.1
<i>R. lunulatus</i>	2	0.7
<i>R. zambeziensis</i>	4	1.7
Total	268	

Table 3: Total tick counts according to farming sector and tick species identified by Microscopy

Tick species	Communal		Commercial	
<i>A. hebraeum</i>	Gwanda	52	Grills farm	2
	Nkayi	24	Beitbridge	23
	Mangwe	11	Umguza	30
	Insiza	3		
<i>A. variegatum</i>	Gwanda	1		
	Nkayi	1		
<i>H. truncatum</i>	Gwanda	7	Grills farm	4
	Nkayi	7	Umguza	9
	Mangwe	2		
	Insiza	1		
<i>H. rufipes</i>	Nkayi	2	Grills farm	1
	Insiza	3	Umguza	4
<i>R. evertsi evertsi</i>	Nkayi	12	Umguza	9
	Mangwe	8		
	Insiza	4		
<i>R. (B) decoloratus</i>	Nkayi	8	Umguza	5
	Mangwe	3		
<i>R. appendiculatus</i>	Mangwe	3	Umguza	10
	Insiza	5	Beitbridge	1
			Grills farm	1
<i>R. sanguineus</i>	Nkayi	1		
	Mangwe	1		
	Insiza	1		
<i>R. simus</i>	Insiza	1	Umguza	2
<i>R. lunulatus</i>			Grills farm	2
<i>R. zambeziensis</i>	Nkayi	2	Grills farm	2

The primer produced four polymorphic bands among *Hyalomma truncatum*, *Hyalomma rufipes*, *Amblyomma hebraeum* and *Rhipicephalus lunulatus* from 600bp to 400bp. The primer also produced one band of 1500bp between *Hyalomma rufipes* and *Rhipicephalus lunulatus*. Monomorphic bands from 1200bp to 800bp were also shared among the four taxa with the same primer. OPO-05 and OPX-14 revealed

DISCUSSION

A total of 268 ticks were collected during the study and identified to belong to 11 species and three genera, namely *Amblyomma*, *Rhipicephalus* (including the subgenus *Boophilus*) and *Hyalomma*.

Of all the ticks that were collected, male ticks account for 59% (159) and females 41% (109). The percentage of male ticks was significantly higher than female ticks. The higher proportion of males could be partly due to the reproduction behavior of ticks; whereby male ticks remain on the host and will attempt to mate with many females whilst they are feeding whereas the females mate only once, before they are ready to engorge fully with blood and detach from the host with enough sperm stored to fertilize all their eggs (Walker *et al*, 2014).

Of all the sampled sites, only Beitbridge had a tick infestation ration below 50%. This condition indicates that there is inadequate usage or improper usage of acaricides in Zimbabwe as compared to the cattle imported from Botswana which had a significantly lower infestation rate compared to that of Zimbabwean cattle $x^2(P<0.02)$.

The prevalence of tick infestation in commercial farms was also higher than expected as suggested by Ndhlovu *et al.*, (2009), Grills farm having the highest infestation rates (56%). Commercial farming sectors are also recording high rates of tick infestation (Table 3) which suggest two

more distinguishing than shared genetic characters as was shown by the presence of more polymorphic bands than common bands. OPO-05 produced six polymorphic bands among the four taxa from 1000bp to 600bp. More distinguishing bands were also observed from 1500bp to 1200bp with the same primer (Supplementary Figure 1). *Hyalomma truncatum* was not at all amplified by OPX-14.

possibilities; either the farmers are improperly using acaricides or ticks are developing acaricide resistance.

If the former is true then farmers must be advised on the proper use of acaricides. If the latter is true then the matter remains critical as acaricide resistant ticks can cause heavy economic losses unexpectedly (Abbas *et al.*, 2014). Out of the tick infested Botswana cattle, none had a tick species novel to Zimbabwe. The ticks from Botswana imported cattle belong to the two species *Hyalomma truncatum* and *Hyalomma rufipes* which are already present in Zimbabwe. Nevertheless the imported cattle will need to be monitored from time to time so that the Zimbabwean Government is on the alert. It was also noted that male ticks occur more frequently (59%) than female ticks on cattle.

Amblyomma hebraeum had the highest occurrence and widest distribution in the region (54%) both in the communal farming sector and the commercial farming sector (Table 2).

The findings are in consensus with those of Ndlovu *et al.*, (2009) that *Amblyomma hebraeum* has a higher prevalence in the lowveld areas. The occurrence of *Amblyomma variegatum* in this part of the country nevertheless disagrees with the findings of Peter *et al.*, (1998a) as well as the findings of Ndhlovu *et al.*, (2009). According to these authors, the northern

limit of *A. hebraeum* is latitude 17° south, while north of this limit it is replaced by *A. variegatum*. Peter *et al* (1998a) cited in Ndhlovu *et al.*, (2009) also reported that *A. hebraeum* was predominant in the lowveld and had encroached into some parts of the highveld of Zimbabwe yet in this study *A. variegatum* was reported in the lowveld both in Matabeleland South and Matabeleland North.

Earlier on the presence of the *A. variegatum* was limited to the Zambezi valley and the surrounding dry lowveld areas in the north-western parts of Zimbabwe (Norval *et al.*, 1994) thus its presence was quite unexpected in Matabeleland. Its presence in Nkayi can to a certain extent be explained by the closeness of Nkayi to the Northern parts of Zimbabwe but its presence in Gwanda remains unexplained.

It can thus be proposed that its presence in Gwanda is due to the migration of farmers across the country perhaps due to the recent land reform program. The two species have been reported to have an area overlap where mating between the two results in sterile offspring. Taking note of the fact that *A. variegatum* has of now been reported in the southern parts of the country in this study; there are therefore a number of possibilities.

A. variegatum is known to displace *A. hebraeum* in a period of three years (Ndhlovu *et al.*, 2009) thus it is possible that the latter will be replaced in the southern parts of the country or a novel precedence may be set where the two species coexist in the same geographical zone. The two species had predilection sites that spread all over the bodies of cattle.

These findings also agree with those of Norval *et al*, (1994) as the *Amblyomma* species can feed from any part of cow bodies. The findings of this study showed that the *Hyalomma* species co-exist in the same ecological zone. *Hyalomma truncatum*

(11%) however had a higher occurrence rate than *Hyalomma rufipes* (3.7%). The findings are in agreement with observations by Horak *et al.*, (2001) who reported that the two species can overlap in the same ecological zone.

The two *Hyalomma* species also have the tail switch as their predilection site over other sites. This could be due to the fact that the tail switch has a lot of hair that can camouflage the ticks from birds seeking to feed on them as the *Hyalomma* species are among some of the largest ticks.

Rhipicephalus (Boophilus) decoloratus has been documented to occur in most of the country. The absence of the close relative *R. (B) microplus* was expected, as it has a limited distribution in Zimbabwe.

On the other hand, the distribution of *R. (B) microplus* is limited in the eastern region, northern, north-eastern and the north-western region of Zimbabwe (Katsande *et al.*, 1996).

Rhipicephalus appendiculatus and *Rhipicephalus zambeziensis* are closely related and have been documented to co-exist. The occurrence of *R. appendiculatus* (7.4%) was higher than *R. zambeziensis* (1.7%) in the region. This could probably be explained by the fact that *R. appendiculatus* has been documented to have a competitive advantage over *R. zambeziensis* in cooler and more humid conditions whereas *R. zambeziensis* has a competitive advantage in hotter and drier conditions (Ndhlovu *et al.*, 2009).

Rhipicephalus evertsi evertsi (12%) had the highest occurrence in the region among the *Rhipicephalus* species. Its relative *Rhipicephalus appendiculatus* had a lower prevalence (7.4%).

Ndhlovu *et al* 2009 reported that *Rhipicephalus appendiculatus* had the

highest rate among the *Rhipicephalus* species in Matabeleland South. The results from this study therefore suggest that *R. e. evertis* is more distributed in Matabeleland North (64%) than in Matabeleland South (36%). *Rhipicephalus sanguineus* (1.1%), *Rhipicephalus simus* (1.1%) and *Rhipicephalus lunulatus* (0.7%) had very low prevalence rates in the region. These ticks also had the tail as their main predilection site. These results agree with Van Niekerk *et al.*, (2006) who reported similar findings. Van Neikerk *et al.*, (2006) also suggested that high occurrence of these tick species in the tail switch is due to poor dipping method where farmers do not thoroughly wet the tail switch or ignore it completely, then these ticks would thrive.

From the results acquired during DNA extraction in this study it can be argued that ethanol preservation can interfere with tick DNA extraction process (30%). Thus fresh tick samples (75%) are most favorable to use during tick genetic studies. This statistic was also supported by Cruickshank, (2002). Ethanol preserved samples must be soaked in sterile water overnight before processing can commence as this extraction yield from preserved samples. Three primers were able to amplify the template DNA, that is, OPY-03 OPX-14 and OPO-05. OPY-03 was the primer of choice as it was able to amplify all samples.

Common bands (500bp-800bp) could be observed for *Hyalomma truncatum* and *Hyalomma rufipes* for genetic fragments of OPY-03 (Supp. Figure 1), indicating the close genetic relationship between the two as they are from the same genus.

Monomorphic bands from 1000bp to 800bp of OPY-03 genetic fragments shared among the four taxa indicated highly conserved

regions among the three genera.

Similar amplification is also observed specifically in OPO-05 fragments of 1500bp suggesting conserved regions in the three genera. *Amblyomma hebraeum* and *Rhipicephalus lunulatus* showed a high number of polymorphic bands for genetic fragments of OPO-05 from 1100bp to 800bp showing a distant relationship between the two species as they belong to different genera.

Considering difficulties encountered in accurate morphological identification of ticks, RAPD analysis has improved tick identification. RAPD-PCR primers have been successfully used for rapid detection of Iranian tick species; *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum* (Abdigoudarzi *et al.*, 2004).

The variability produced by the RAPD profiles for the three primers used in this study suggests the existence of genotypic diversity among the various isolates of ticks used in this study. RAPD analysis was therefore used for the first time in Zimbabwe to characterize the four species.

CONCLUSION

Eleven tick species occur in Matabeleland region, though somewhat an unexpected finding as it is rare to find more than eight tick species occurring one geographical zone. Remarkably identified also is the occurrence of *A. variegatum*, a known northern tick, in the southern part of the country. The RAPD analysis in this study showed differences among four tick species. Further research must therefore be carried out on this subject to increase our taxonomic understanding of ticks occurring in Zimbabwe.

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CONFLICT OF INTEREST

Authors do not have any conflict of interest.

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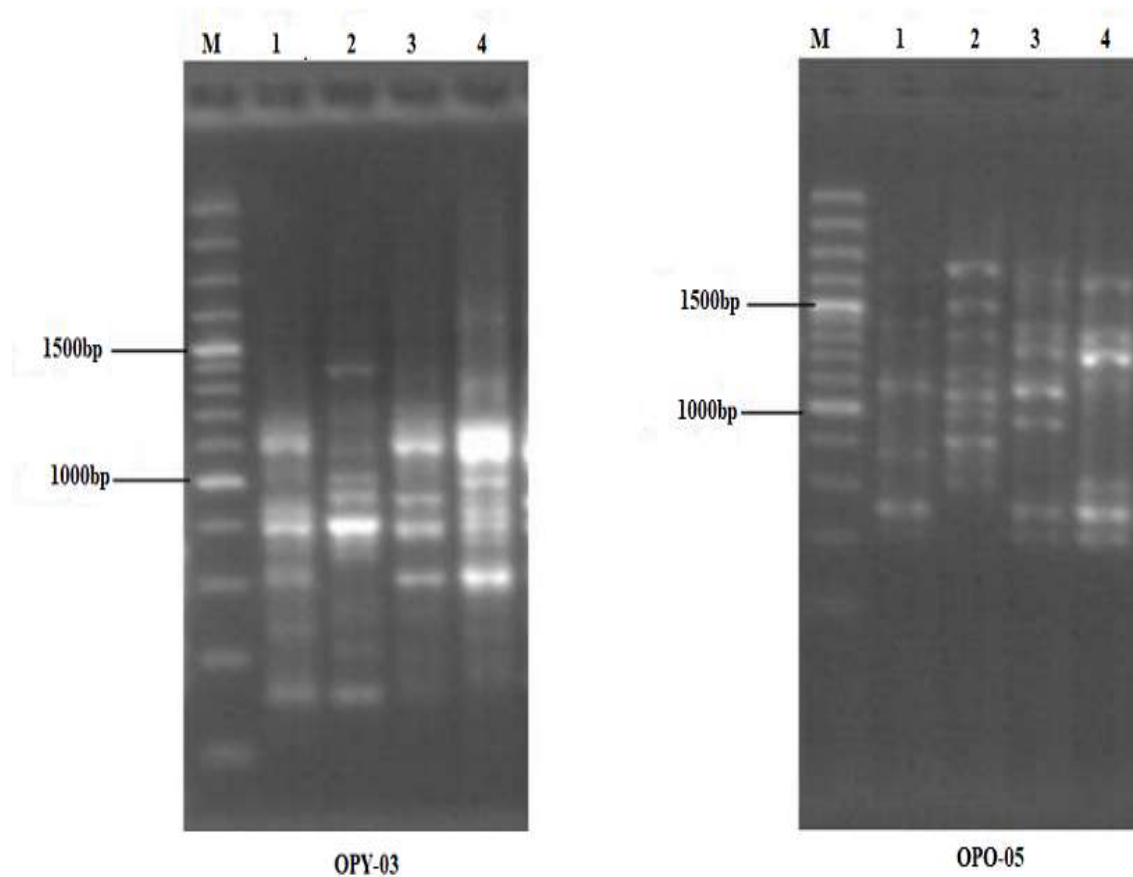
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SUPPORTING INFORMATION



Supplementary Figure 1. Gel image showing RAPD-PCR products using primer OPY-03 and OPO-05 on samples 1- *Hyalomma truncatum*, 2- *Hyalomma rufipes*, 3- *Amblyomma hebraeum*, and 4- *Rhipicephalus lunulatus*. M is a 1kb molecular marker. Four polymorphic genetic fragments of OPY-03 were observed among *Hyalomma truncatum*, *Hyalomma rufipes*, *Amblyomma hebraeum* and *Rhipicephalus lunulatus* from 600bp to 400bp. OPY-03 also produced one common band of 1500bp between *Hyalomma rufipes* and *Rhipicephalus lunulatus*. Monomorphic bands from 1200bp to 800bp were also shared among the four taxa with OPY-03. OPO-05 produced six polymorphic bands among the four taxa from 1000bp to 600bp. More distinguishing bands were also observed from 1500bp to 1200bp with OPO-05. Common 1000bp genetic fragments of OPO-05 were also observed among the four taxa used in this study.