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Short communication

Population genetic structure of *Ascaridia galli* of extensively raised chickens of South Africa

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ABSTRACT

Ascaridia galli is one of the most common nematode affecting chickens. This study characterized *A. galli* parasites collected from South African village chickens of Limpopo ($n = 18$) and KwaZulu-Natal ($n = 22$) provinces using the 510 bp sequences of cytochrome C oxidase subunit 1 gene of the mitochondrial DNA. Fourteen and 12 polymorphic sites were observed for Limpopo and KwaZulu-Natal sequences, respectively. Six haplotypes were observed in total. Haplotype diversity was high and ranged from 0.749 for Limpopo province to 0.758 for KwaZulu-Natal province isolates. There was no genetic differentiation between *A. galli* from Limpopo and KwaZulu-Natal provinces. The six South African haplotypes were unique compared to those published in the GeneBank sampled from Hy-line chickens raised under organic farming in Denmark. The utility of cytochrome C oxidase subunit 1 gene as a potential genetic marker for studying *A. galli* in village chicken populations is presented.

1. Background

Ascaridia species are among the most prevalent and pathogenic parasitic nematodes found in domestic and wild birds that have a worldwide distribution (Abdelqader et al., 2008). *Ascaridia galli* damages the intestinal mucosa leading to blood loss and secondary infection and occasionally the obstruction of the small intestines of chickens that occurs due to high worm burdens (Soulsby, 1982). Information on the genetic diversity and population structure may be of importance to understanding the genetic properties influencing pathogenicity and response to treatment regimes of any disease pathogen, as well as in tracing of the escalating spread of drug resistance among parasitic nematodes (Kaplan, 2004).

Mitochondrial DNA sequences have been useful markers in studies of genetic variability and population structure in livestock (Muchadeyi et al., 2008) and animal pathogens (Walker et al., 2007). Mitochondrial genomes evolve 5–10 times faster than nuclear genomes (Brown et al., 1982) probably due to lack of replication repair mechanism (Clayton, 1982). They are therefore suitable for discriminating closely related organisms (Kaplan, 2004; Le et al., 2002) especially at the species and sub-species levels (Galtier et al., 2009; Li et al., 2008). Networks analysis of mtDNA sequences has been widely used in phylo-geographic analysis (Bandelt et al., 1999).

Majority of South African chickens are raised extensively by smallholder farmers under low-input scavenging systems and chickens are therefore exposed to parasites and other disease pathogens during scavenging. The type and level of parasitic infections vary across geographic locations which makes it imperative, for disease control, to characterize parasites from different production systems. Traditional control of gastrointestinal nematode infections depends on the use of anthelmintics (Woolaston and Baker, 1996), which are often unavailable in low input production systems. Genetic control strategies that depend on the use of natural resistance and tolerance of host chicken populations to prevailing parasites could be an alternative for disease control in village chicken production systems (Lamont, 1998). However, the success of genetic control strategies depends on a good understanding of the pathogens. This study used *Cox*1 gene of the mitochondrial DNA as a genetic marker to investigate the genetic variability of *A. galli* parasites collected from village chickens in South Africa.

2. Methods

A total of 144 non-descript village chickens kept by smallholder communal farmers were sampled from two geographically

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distinct provinces of Limpopo ($n = 99$) and KwaZulu-Natal ($n = 45$) in South Africa The distance between the villages were between 25 and 160 km and 20 and 140 km in Limpopo and KwaZulu-Natal provinces, respectively. Chickens were slaughtered by cervical disarticulation and the gastrointestinal tracts were removed according to the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines (Yazwinski et al., 2003). Briefly, the whole intestine was removed and placed in a tray. It was then opened in a longitudinal section with a pair of scissors from the gizzard to the cloaca. The intestinal contents were recovered and gastrointestinal parasites visible with a naked eye were identified based on the morphological parameters using the helminthological keys (Soulsby, 1982).

DNA was extracted from adult *A. galli* worms using QIAamp blood and tissue kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was performed using primers (G*Cox*1F4F, 5-ATTATTACTGCTCATGCTATTTTGATG and G*Cox*14R, 5-CAAAACAAATGTTGATAAATCAAAGG) (Katakam et al., 2010). PCR amplicons were directly sequenced using the same primers on ABI3500 XL genetic analyzer at Inqaba-Biotec, Pretoria (South Africa). Sequence data were assembled and edited using GAP4 of the Staden package (version 1.6.0) (Staden et al., 2000) and resulted in 510 bp consensus sequences. The sequences were aligned with the available sequences of related genera downloaded from the GenBank (Supplementary Table 1) using ClustalX version 2.1 (Larkin et al., 2007). The haplotype sequences produced in this study were deposited into GenBank with these accession numbers: KT388435- KT388440.

The *Cox*1 gene sequences diversity indices were determined using DnaSP software version 5.10.01 (Rozas et al., 2003). The level of differentiation within and among populations was estimated using analysis of molecular variance (AMOVA) implemented in ARLEQUIN v3.5 (Excoffier and Lischer, 2010). Gene flow between populations was estimated based on the F_{ST} derivative of migration ($Nm = (1/1 + 2Nm)$) over 1000 simulations using DnaSP 5.10.01 (Rozas et al., 2003; Wright, 1951). The Tajima's D (Tajima, 1989) and Fu's Fs tests (Fu, 1997) were also performed using DnaSP 5.10.01 for mismatch distributions of exponential population growth.

The forty sequences from Limpopo and KwaZulu-Natal provinces together with four partial *Cox*1 *A. galli* sequences from the GeneBank were aligned and trimmed to produce overlapping sequences that were 393 bp long. NETWORK 4.1.0.8 (Fluxus Technology Ltd.) was used to generate intra-species median-joining networks and investigate the possible evolutionary relationships among all sequences of each identified haplotype (Bandelt et al., 1999).

3. Results and discussion

Twenty-nine (64.44%) of the 45 chickens slaughtered in KwaZulu-Natal province and 36 (36.36%) of the 99 chickens slaughtered in Limpopo province were positive for one or two adult parasite species. A total of 201 and 228 parasites were recovered from Limpopo and KwaZulu-Natal provinces, respectively. In Limpopo province, tapeworms had the highest prevalence of 54%, followed by *A. galli* and *Heterakis gallinarum* at 32% and 13%, respectively. The prevalence of *A. galli* was 72% and that of tapeworm 27% in KwaZulu-Natal province. The average intensity of infection for *A. galli* in KwaZulu-Natal province was highest with average worm count of 7.2 ± 13.76 per chicken whilst that for Limpopo was 3.42 ± 2.34. A detailed analysis of prevalence and worm burdens in the two provinces was presented by Malatji et al. unpublished. Overall, 40 *A. galli* parasite worms sampled from chickens from Limpopo ($n = 18$ chickens) and KwaZulu-Natal provinces ($n = 22$ chickens) were sequenced.

Table 1
Sequence diversity indices of *Cox*1 gene of mtDNA in Limpopo and KZN *A. galli* parasite populations.

Population	*N*	*S*	*H*	Hd	*Π*	*k*
Limpopo	18	14	6	0.749	0.013	5.150
KZN	22	12	4	0.758	0.014	5.307

N: Number of sequence used; *S*: Number of segregation site; *H*: Number of haplotypes; Hd: Haplotype diversity; *Π*: Nucleotide diversity; *k*: Average number of differences.

The 40 sequences were closely related to *A. galli* mitochondrion, complete genome (Accession no. JX624728), *A. galli* haplotype I (Accession no. GU138668), *A. galli* haplotype II (Accession no. GU138669), *A. galli* haplotype III (Accession no. GU138670), and *A. galli* partial *Cox*1 gene (FM178545) with percentage identity ranging from 97% to 99%. Polymorphisms were observed amongst the samples from different geographical localities as illustrated in Supplementary Fig. S1.

Fourteen polymorphic positions were observed, which defined 6 haplotypes (Table 1). Haplotype diversity (Hd) of the two populations was moderate for both provinces and ranged from 0.749 for Limpopo province to 0.758 for KwaZulu-Natal province (Table 1). The average nucleotide diversity pi (π) was 0.013 with an average number of nucleotide differences of $k = 5.213$ between populations. Haplotype diversity was higher compared to that observed in other *Ascaris* of pigs and humans. A lower haplotype diversity ranging from 0.138–0.605 was reported for *Ascaris lumbricoides* and from 0.553–0.741 for *Ascaris suum* in China (Zhou et al., 2011).

The AMOVA analysis indicated no significant genetic differentiation among the populations (P-value = 0.584). The genetic variation within and among populations was 0.379 and 0.006, respectively. The genetic differentiation was mainly observed within populations. A low fixation index (F_{ST}) value of 0.017 (P-value = 0.584) was observed that indicated no differentiation between the overall population and its subpopulations. Absence of population substructure may be an indicator of common maternal origin of parasites from different provinces. This observation was different from what was observed with Swedish and Danish *A. galli* populations that were moderately sub-structured according to farms and geographical areas (Höglund et al., 2012) despite the higher geographic distances between the South African provinces sampled (>700 km) compared to that between Swedish farms that ranged from approximately 125–324 km. However, genetic diversity within population was higher in the current study than reported in previous studies where most of the genetic variation between the nematodes was contained within individual Jönköping and Danish hens (Höglund et al., 2012).

The *A. galli* sequences had a positive Fu's Fs value of 0.625 ($P = 0.016$) and Tajima's D value of 1.842 ($P > 0.05$). Mismatch distribution analysis of the complete datasets revealed the presence of a multi-peak (Fig. 1), which suggests that there was no rapid expansion event that occurred in the South African's *A. galli* population's demographic history and a gene flow (Nm) value of −30.53 was observed. The large and negative Nm value in this study indicated less gene flow among the *A. galli* populations from the two provinces over time suggesting less movement of chickens between provinces. Höglund et al. (2012) observed a relatively high gene flow amongst Swedish *A. galli* isolates in contrast to our findings. The positive values from Tajima's D test signify that *A. galli* might not have experienced population expansion in the past. The Tajima's D and mismatch distribution analysis confirmed that *A. galli* from the two provinces were genetically similar and could not be considered as distinct populations. The high level of genetic similarity between the KwaZulu-Natal and Limpopo populations could therefore be due to other factors other than gene flow such as over-