

## Research Article



# Rationale to Understand Anthelmintic Resistance in Parasitic Nematodes

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**Abstract** | With extensive use of the anthelmintic in many tropical and parasite-endemic countries, the graveness of parasite resistance is exaggerating. The trend in the use of excessive medication in animal is also posing threats to public health. The governmental bodies are either lacking understanding or underestimating the regime of this emerging issue. This brief overview highlights the need, rationale and urgency of the matter to establish foundations for unified action plans at the national levels as well as international levels in mitigating the development of resistance.

**Keywords** | Anthelmintic, Resistance, Nematodes

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Gastrointestinal (GI) round worms are the main concern of the morbidity and production losses in livestock costing the North USA cattle industry alone more than \$2000 million per year (Stromberg and Gasbarre, 2006). Genus *Haemonchus* is the blood sucking roundworm infecting about all ruminants worldwide and produced high economic losses to the large and small ruminant industries. *Haemonchus* have four major species, *H. contortus*, *H. placei*, *H. longistipes* and *H. similis*. There are many debates about the *H. placei* and *H. contortus* relationship, which belongs to a single species. But now it is proved that they are different species on the bases of morphological characteristic and genetic evidence. Morphological difference includes the fixed number of the longitudinal ridges on the surface of cuticle and spicule pattern. The X chromosome size is also different in both species observed during the staining of DAPI metaphase spreads. The size of X chromosome is bigger than the autosome in *H. placei* while in *H. contortus* both types are same size. On the other hand genetic examination of the ITS-II segment of the ribosomal DNA, NTS and NADH subunit 4 gene of the mDNA nucle-

otide sequence reveals that the fixed difference between these two closely related species (Chaudhry et al., 2015a).

Among these three the *H. placei* represent the most important and highly pathogenic species of cattle. The closely related nematode *H. contortus* is extremely high drug resistant parasite in the world which is predominantly a small ruminant parasite (sheep, goats) having significant cost-effective importance in both tropical as well as in sub-tropical areas. Although, *H. contortus* is predominantly sheep and goat parasite, but it also causes disease in many artiodactyl animals including bovine. Same situation is on the other hand the *H. placei* is predominantly parasite of the large ruminant but it also infects small ruminant and also co-infection reported in diverse country of the world (Tan et al., 2014). Many studies are published worldwide on the co-infection among the *H. placei* and *H. contortus* in small ruminants and large ruminants (Achi et al., 2003; Amarante et al., 1997; Brasil et al., 2012; Gasbarre et al., 2009b). In contrast the published research works on the interspecies hybridization between the different roundworms are

few in numbers (Chaudhry et al., 2015a; Criscione et al., 2007). Co-infection between the both species leads to the possibility of interspecies hybridization. It was experimentally proved that the crosses between interspecies lead to the production of hybrid F1. F1 hybrid is sterile but if they mate with parental male it produces the viable offspring, if this happens in the field conditions then it produces introgression of gene (interspecies) which is responsible for the spread of drug resistance mutation among the species (Chaudhry et al., 2014).

The evidence of interspecies hybridization amongst the *H. placei* and *H. contortus* proved that they have potential of introgression of drug resistance gene among these two parasites suggesting this could be a very essential process for the spread of interspecies anthelmintic drug resistant changes (Chaudhry et al., 2014). The molecular genetic analysis of the *H. placei* and *H. contortus* 7 hybrid worms were recognized in previous studies, two hybrid worms from Brazil and 5 hybrids worms from Pakistani sheep and goats (Brasil et al., 2012; Chaudhry et al., 2015b). Genetic basis of the hybridization in cattle and buffalo has not yet been investigated. We analyze the interspecies co-infection, (*H. placei* and *H. contortus*) hybridization and introgression of the isotype-1 $\beta$ -tubulin gene from cattle and buffalo *Haemonchus* populations.

The GI parasitic nematode *H. placei* represents the most important and highly pathogenic species of cattle. The closely related nematode *H. contortus* is predominantly a roundworm of the small ruminants, also infects large ruminant having significant financial importance in hot (tropical) and sub-tropical areas of the different countries (Hoberg et al., 2004; Lichtenfels et al., 1986). The mechanism of benzimidazole resistance has been inspected in small ruminant parasites and solid proof of the replacement of the single amino acid at different position [i.e., F200Y (TAC), F167Y (TAC) and E198A (GCA)] in the isotype-1 gene of the  $\beta$ -tubulin are present which are accountable for the resistance production in benzimidazole, but there is no or limited evidence in large ruminants (Anziani et al., 2004; Chaudhry et al., 2016). In spite of a well-known worldwide concern about the progress of benzimidazole resistance in small ruminant worms, but to date a very low consideration was given to the parasite of large ruminants (Coles, 2002; Jackson et al., 1987). Nonetheless, benzimidazole resistance in large ruminant parasites are now a day developing and represents a thoughtful challenge to the large ruminants industry of the world (Gasbarre et al., 2009a; Sutherland and Leathwick, 2011; Wolstenholme et al., 2004). Only few studies of benzimidazole resistance were performed at molecular level in large ruminates. In a few researches, the genetic factors were reported in emergence of drug resistance. Benzimidazole resistance was recorded in

*Cooperia oncophora*, *Ostertagia ostertagi* field isolates that were related with the F200Y (TAC) and E198A (GCA) mutations (Demeler et al., 2013; Winterrowd et al., 2003). There have been very limited studies of F200Y (TAC) and F167Y (TAC) mutations which are linked to benzimidazole resistance in *H. placei* (Brasil et al., 2012; Chaudhry et al., 2015b). The applications of drugs used for helminths provide strong evidence of positive selection pressure for the adaptive genetic changes in parasitic populations. For understanding of the nature resistance mutations that develop in result of drug selection may helpful for the understanding of the emergence of anthelmintic resistance due to genetic changes (Chaudhry et al., 2014). Firstly, resistance could emerge as a single mutation and then spreads to whole population of parasite by movement of host animal. In the above case a single resistance haplotype would be emerged and swept through the whole population. This has been observed for benzimidazole resistance allele E198A (GCA) in *H. contortus* of small ruminants in Pakistan (Chaudhry et al., 2015b). Secondly, anthelmintic resistance mutations could repeatedly emerge at multiple times and transfer to the other populations of the parasites as a result of host migration. In this case multiple resistance haplotypes may sweep through the populations. This type of sweep has been reported recently for benzimidazole resistance alleles F200Y (TAC) and F167Y (TAC) in *H. contortus* of the small ruminants (Brasil et al., 2012; Chaudhry et al., 2015b; Redman et al., 2015; Silvestre and Humbert, 2002). In contrast, there is lack of information about the understanding of the benzimidazole resistance emergence and the spread in large ruminants *Haemonchus* worms.

In our study, we explore the incidence of F200Y (TAC) resistance genetic mutations in  $\beta$ -tubulin isotype-1 locus of seven *H. contortus* and ten *H. placei* populations of buffalo and cattle. Furthermore, the phylogenetic analysis of *H. placei* populations has been performed to look for the evidence of the emergence of single F200Y (TAC) mutation suggesting the spread of resistance allele from one location to another through gene flow. According to our information, this is the first evidence on the bases of genetic analysis of the emergence of F200Y (TAC) resistance in *H. placei* from a single mutation in bovine host from Pakistan. In contrast, the F200Y (TAC) mutation recorded as multiple independent emergences more frequent occurrence in *H. contortus*.

Nematode parasite of the superfamily *Trichstrongyloidea* carry high rate of genetic diversity which recognized to the effective size of the large population and extraordinary rates of genetic mutation (Gilleard and Beech, 2007; Hunt et al., 2008; Prichard, 2001). In small ruminants, *H. contortus* has high rates of genetic diversity that contributes the large size of the parasitic population (Chaudhry et al.,

2015b; Redman et al., 2015; Urquhart et al., 1996). Many studies have inspected that in what way population molecular structure is divided parasitic nematodes in different category (Ali et al., 2014; Redman et al., 2015). Previous experiments in the world for the molecular analysis of *H. contortus* in small ruminant showed a great level of genetic differentiation between the countries, through the population from any one fundamentally making monophyletic groups. Likewise, much more intensities of the genetic difference were observed among the laboratory strains of the *H. contortus* collected from the different areas (Redman et al., 2015). Above information is not shocking due to inadequate potential of gene movement. First experiment on the field isolates of *H. contortus* in United States of America tells us that there was no principally genetic variation essentially inside the North USA due the very big effective herds sizes collective with huge gene movement by the movement of animal (Blouin et al., 1995). Nonetheless, following studies by means of extra differential genetic markers reveals that comparatively little amount, however important level of local molecular genetics differentiation present among the *H. contortus* in different countries (i-e) Pakistan, United Kingdom, Australia, Sweden and France (Ali et al., 2014; Hunt et al., 2008; Redman et al., 2015; Silvestre and Humbert, 2002). *H. contortus* populations in all above countries and United Kingdom were shown to have particular genetic differentiation where huge animal movement was existed (Redman et al., 2015). Population dynamics of parasitic nematodes have been studied for the understanding of complex epidemiology involving free living parasitic stages, effects of environment, management of animal and responses of parasite to host (Yin et al., 2016). *H. contortus* in small ruminants is the example, in which the most larvae died due to the environmental conditions (very low temperature) during the winter season in United Kingdom and Sweden, or dry and burning times during the summer season of Australia.

In summary, the parasites which are survived in these both hot and cold weather conditions are undergone the hypobiosis in the form of 4<sup>th</sup> stage larvae inside their hosts. Consequently, the chance of bottlenecking in parasitic population increases which ultimately decreases the genetic variation as well as genetic diversity in these areas.

Parasites that are biologically same are frequently isolated by their presence in various host species or then again unique areas in a similar host. From now on, two sympatric phylogenetic origin related worm species with a same geographic spreading may not ever get to mate each other. But this is not true in case of *Haemonchus* species who share the stomach for living in their ruminant hosts. In spite of the way that, there is very limited information on the history of the *H. placei*, but normally it is recognized as a parasite of cattle. On the other hand *H. contortus* is regularly found in

small ruminants, it also causes morbidity in different artiodactyl animals (Hoberg et al., 2004), containing household ruminant species, for example, dairy cattle (Hogg et al., 2010). In addition, *H. placei* and *H. contortus* coinfection was reported in small ruminants (Amarante et al., 1997; Chaudhry et al., 2015a). In spite of fact that it is indistinct how generally this happens in dairy cattle. The possibility of hybridization increases due to the co-infection of these two more phylogenetically related species. Hybridization between firmly related species is ordinary in plants, however is regularly seen as more bizarre in animal. Be that as it may, there is an expanding acknowledgment that hybridization occurs more normally between the sympatric animal species in environment than before-hand perceived (Jacquet et al., 1998). Hybridization between species is constantly uncommon for every individual basis, yet this announcement is redundant on the grounds that species hybridization was regular between specific gatherings of animal they would not be perceived as various species (Mallet, 2005). There have been not very many examinations to research interspecies hybridization between various trichostrongyloid nematode parasites of livestock. Mostly the research work of co-infections and hybridization was performed in nematode parasite of the trichostrongyloidea super family after detailed morphological examination to recognize possibility of hybrids (Le Jambre, 1979). Main research work for the study of hybridization has been performed on *H. contortus*/*H. placei* /*O. ostertagei* /*O. leptospicularis* in sheep, *C. onchophora* /*C. pectinata* in cattle; for every circumstance solid affirmation for exploratory hybridization between the species has been reported in above species set (Isenstein, 1971; Le Jambre, 1979; Suarez et al., 1993). After a short time later, it is possible that hybridization is a normal phenomenon between the species in field condition, through which it transfers the anthelmintic resistance mutation among the diverse ruminant trichostrongyloidea nematodes species. Regardless the way that the cattle are the main host of *H. placei* (Lichtenfels et al., 1986), the other sympatric species the *H. contortus* can infect many other artiodactyl host but most commonly sheep and goats (Hoberg et al., 2004; Lichtenfels et al., 1994; Lichtenfels et al., 1986). In field conditions mostly the *Haemonchus* infections were diagnosed at genus level, very few data is available at species level so that information's about the species and co-infection are very little. Few peer-reviewed data is available on the *H. placei* presence in sheep, goats and presence of *H. contortus* in dairy cattle (Akkari et al., 2013; Hogg et al., 2010). Although, co-infection between these two species in sheep and goats was also reported (Achi et al., 2003; Amarante et al., 1997; Brasil et al., 2012; Jacquet et al., 1998). The presence of the two closely related species of *Haemonchus* was reported in different countries which indicated the co infection and hybridization was more prominent in small ruminants than large ruminants (Chaudhry et al., 2015a). There are also a number of pub-

lished reports of presence of hybrids in small ruminant in natural field conditions stimulating the proposal of hybridization (Brasil et al., 2012; Chaudhry et al., 2015a; Lichtenfels et al., 1994; Lichtenfels et al., 1986). *H. contortus* and *H. placei* co-infection happens again in many ruminants under characteristic field that could be a possibly critical source. There are very few reports of co-infection of *H. placei* and *H. contortus* in cows and not a single reported in the buffalo (Gasbarre et al., 2009a; Gasbarre et al., 2009b).

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