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RNA interference studies in helminths of veterinary importance: A review

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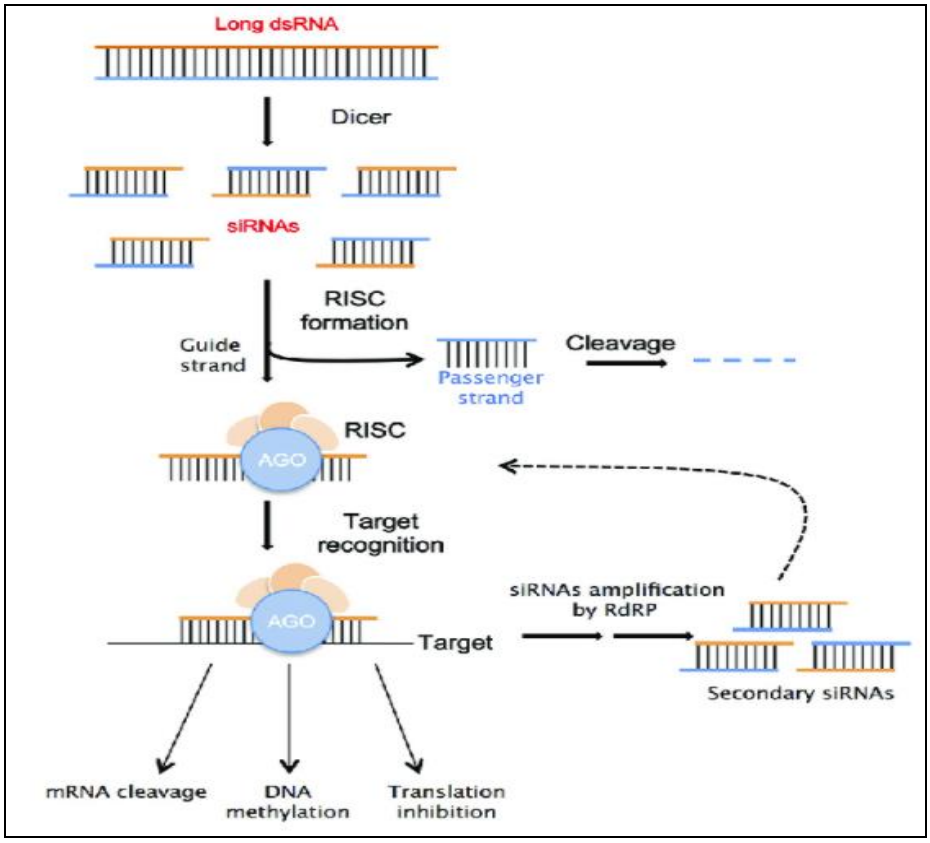
Abstract
With the advent of method of RNA interference (RNAi) in the era of prompt diagnosis and therapy of parasitic diseases caused by helminths has paved way to newer platforms. Various researchers have performed there studies on different trematodes like *Fasciola hepatica, Fasciola gigantic* and various nematodes viz; *Ascarissuum, Haemonchus contortus, Ostertagia ostertagi, Trichostrongylus colubriformis* etc. with considerable success. But still, the method needs to be validated further in absence of lack of consistency and reproducibility. Certain off-targets effects needs to be minimized. To combat drug resistance, the method of RNA interference stoods as an alternate approach to prevent parasitic diseases.

Keywords: RNA interference (RNAi), diagnosis, alternative approach, therapy of parasite

Introduction
The RNAi method includes the study of gene modifications and functions hence seek the possibility to control disease pathogens or vectors in the field of veterinary sciences. RNAi previously known as gene silencing, this method employs introduction of a double stranded RNA (dsRNA) into the organism, which targets the desirable mRNA template and reduces its expression. It was first discovered in *Caenorhabditis elegans* by Craig Mello and Andrew Fire in 1998, and thereafter applied to various other parasites. The technique involves various components like dsRNA, dicer, short interfering (siRNA), argonaute family of proteins, RNA dependent RNA polymerases enzyme (RdRp) and other protein molecules like systemic RNA interference-deficient (SID-1, SID-2) and RNAi spreading defective (RSD-4) proteins [1]. It works on a mechanism of gene-silencing including introduction of dsRNA, which can be an exogenous, viral particle or transposon into the host cell or organism. RNAi is multidimensional, and there are various pathways in which small double-stranded RNA (dsRNAs) control gene expression. Hence, RNAi gives us a better insight in understanding gene functions in parasites; find better drug targets and vaccine candidates [2].

Mechanism of action of RNAi
In the various studies made by researchers it was evident that a dicer molecule, which is a ribonuclease III enzyme cleaves the dsRNA into fragments of size 22bp, known as siRNA, having high binding affinity for the target mRNA template. Binding of siRNA, activates the RNA induced silencing complex (RISC complex), which leads to degradation of the homologous RNA affecting the phenotypic gene expression. There are number of techniques available by which dsRNA is introduced into the cell like electroporation, soaking of the worm into the solution containing dsRNA [3] or by feeding worms on bacteria expressing dsRNA [4] or by microinjection [5]. Uptake of dsRNA into the cell is facilitated by transmembrane proteins SID-1 which forms channels in the membrane to enables diffusion of dsRNA [6] and SID-2 which is located in the intestine of the worm and is involved in the uptake of exogenous dsRNA [7] The introduction of double-stranded RNA (dsRNA) changes only the phenotypes of organisms without modifying the genotypes [8]. The dsRNA reduces the transcripts of specific mRNA, instead of deleting or inserting a gene unlike genetic modifications.



Long dsRNA

RNA interference studies in various helminth parasites of veterinary importance

RNA interference method can be wisely used in most of helminth parasites of veterinary importance viz., *Fasciola hepatica*, *Fasciola gigantica*, *Ascaris suum*, *Haemonchus contortus*, *Ostertagiao stertagi*, *Trichostrongylus colubriformis* etc., to achieve diagnostic as well as therapeutic benefits. *Fasciola gigantica* and *F. hepatica* are the two species causing fasciolosis in sheep, goat, cattle and other ruminants. The drug of choice being triclabendazole has been used indiscriminately leading to increase incidences of anthelmintic resistance. Thus, to suppress such side-effects RNAi approach is successfully applied [9, 10] While working on *Fasciola hepatica*, targeted a single copy gene, encoding for leucine aminopeptidase (LAP) as a model to refine delivery conditions. Electroporation was found to be an efficient mode for introduction of small RNAs into the fluke [11]. Initial report highlights the role of cysteine proteases in gut penetration by the newly excysted juveniles of *Fasciola hepatica* by applying method of RNAi. The three genes of commercially-available liver fluke strain (the US Pacific North, West, Wild Strain) were optimized, namelycathepsin L (FheCatL) and B (FheCatB) cysteine proteases and a s-class glutathione transferase (FhesGST). A tedious transcriptional silencing of targets in both *F. hepatica* and *Fasciola gigantica* juveniles was achieved following exposure to long (200–320 nt) dsRNAs or short interfering (27 nt) (si)RNAs. In spite of marked variation in silencing, it was found that a transient exposure to long dsRNA or siRNA triggers robust RNAi penetrance and persistence in liver fluke newly excysted juveniles, supporting the development of multiple throughput phenotypic screens for control target validation [12]. Anandanarayanan and co-workers [13] optimized RNAi in *F.gigantica*, targeting six genes including superoxide dismutase (SOD), σ class of glutathione-s-transferase (GST), cathepsin (Cat) L1-D, Cat B1, Cat B2 and Cat B3, these are antioxidant enzymes that that would suppress oxidative killing of the parasite by the host effector cells. Transcriptional silencing of the targets were achieved when the newly excysted juveniles (NEJs) were exposed to long (170-223 nt) dsRNA. In the above experiment delivery of the long dsRNA and siRNA to the newly excysted juveniles by soaking method was found to be efficient. McCammick [14] focused on the role of Calmodulins (CaM) in *Fasciola hepatica*. RNAi process helps in deduction of underlying fundamental processes of CaM including the phosphorylation of protein kinases, gene transcription, calcium transport and smooth muscle contraction.

Nematode parasites are a major cause of disease in animals and the worldwide economic impact of nematode parasites to the livestock industry is estimated to be more than 10 billion per annum[15]. *Ascaris suum* is a gastrointestinal parasite of pigs which causes significant economic losses in pig industry by having deleterious effects on their health leading to reduce weight of finish swines, poor feed conversion ratios and degraded carcass quality. Microinjection method employed for the induction of RNAi in adult *A. suum* through the injection of double-stranded RNA into the pseudocoelomic cavity of female worms [16]. It was clearly demonstrated that adult *A. suum* is RNAi competent and the induction, spread and consistency of RNAi occurs across multiple tissue types. *Haemonchus contortus* is an important trichostrongylid nematode affecting small ruminants and a cause of significant economic losses due to high mortality and morbidity of animals worldwide [17]. Matrix metalloprotease 12A (MMP-12) plays important role in embryonic development and morphogenesis of vital organs in animals. siRNA-mediated silencing of Hc-MMP-12 gene in *H. contortus* significantly reduces the egg counts, larval hatchability and adult worm counts and sizes[18]. Samara singh [19] for the first time

determined in-vivo effects of H11. They peformed RNAi silencing of genes encoding for the gut aminopeptidase H11, which ishighly protective, Hc-ASP-1, asecretory protein, β-tubulin and homologues of RNA helicaseand aquaporin. In infective larvae RNAi of the H11 gene prior to infection resulted in reduction of faecal egg count (FEC), worm burden and decrease in aminopeptidase activity when compared with pre-soaking in control dsRNA. Dim-1 is a member of the disorganized muscle family of *H. contortus*. Effective silencing of Dim-1 in third stage larvae (L3) led to reduced L3 migration and slowed larval development from L3 to early L4. Thus, potentiating the therapeutic effect against *H. contortus* [20].

*Trichostrongylus colubriformis*a sheep nematode parasite was tested for the efficiency of RNA interference (RNAi) delivery to L1 through L3 stage of worms [21]. Here, ubiquitin and tropomyosin were considered as a target gene, which is attributed to their conserved nature. Electroporation was found to be efficient in inducing dsRNA. In *Ostertagia ostertagi*, a parasitic nematode of cattle,eight genes were tested by electroporation and soaking delivery methods in their L1 and L3 larval stages. Substantial reduction of transcripts was detected for five target genes (tropomyosin, β-tubulin, ATPase, superoxide dismutase and a polyprotein allergen) in L3 larvae, but dsRNAs of a transthyretin-like protein, a 17 kDa ES protein and ubiquitin did not reduce the target gene transcript levels [22]. In the absence of viable, commercially available vaccines, the control of nematode parasites relies profoundly on a limited collection of chemotherapeutic drugs in veterinary medicine [23]. Significantly, multi-drug resistance has been reported in many of the key parasitic nematodes of livestock [24]. Hence, RNA interference (RNAi) method proves to be an appealing reverse genetics tool. Now days its utility for the validation of appropriate drug targets in veterinary parasites is widely accepted. Standardized RNAi approaches for different helminths have undermined the development of robust gene silencing platforms [25].

Conclusion

The various off-target effects like over-dose lethality, toxic effects and electro- shock must be taken care of and appropriate measures like careful titration should be adopted to minimize them. Nowadays, due to increase pressure of anti-parasitic drug resistance and lack of vaccines in the field of veterinary science, the process of RNAi has come up with a ray of hope, which needs to be further exploited, to be used as a consistent method for identifying a novel molecule for diagnostics or as a therapeutic. The prerequisite is the sequencing of whole genome, so that different function of each gene can be explored. Hence, effective gene silencing can be achieved.

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