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MINI-REVIEW

Molecular tools-advances, opportunities and prospects for the control of parasites of veterinary importance

Sachin Kumar[1,2] · Snehil Gupta[3] · Aquil Mohmad[1] · Ashutosh Fular[1] · B. C. Parthasarathi[1] · Ashok Kumar Chaubey[2]

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Abstract

The recent advancement in genome sequencing facilities, proteomics, transcriptomics, and metabolomics of eukaryotes have opened door for employment of molecular diagnostic techniques for early detection of parasites and determining target molecules for formulating control strategies. It further leads to the introduction of several purified vaccines in the field of veterinary parasitology. Earlier, the conventional diagnostic methods was entirely based upon morphological taxonomy for diagnosis of parasites but nowadays improved molecular techniques help in phylogenetic study and open an another area of molecular taxonomy of parasites with high precision. Control measures based upon targeting endosymbionts in parasites like *Dirofilaria immitis* is also under exploration in veterinary parasitology. Metagenomics have added an inside story of parasites bionomics which have created havoc in human and animals population since centuries. Omics era is playing a key role in opening the new approaches on parasite biology. Various newer generations of safer vaccines like edible vaccines and subunit vaccines and diagnostic techniques based upon purified immunologically active epitopes have become commercially available against the parasites (helminths, protozoa and arthropod borne diseases). Nowadays, a transgenic and gene knock out studies using RNA interference and CRISPR are also helping in understanding the functions of genes and screening of target genes, which are not available before the advent of molecular tools. Molecular techniques had paramount impact on increasing the sensitivity of diagnostic tools, epidemiological studies and more importantly in controlling these diseases. This review is about the advancements in veterinary parasitology and their impact on the control of these pathogens.

Keywords Veterinary parasitology · Parasite diagnosis · Molecular tools · Genetic resistance · Vaccines

Introduction

The molecular biology tools are increasingly becoming relevant to deepen the understanding of veterinary parasitology. Their implication for improvement in diagnosis and control measures against the parasites gets highlighted in last three decades due to increased ease and pace of availability of information about parasite genomes and proteomes via various database like EuPathDB, GeneDB, WormBase, HelmCop, PathOD, EPICDB, TrypanoCyc, LeishCyc and many more such database are available on web portal (Aurrecoechea et al. 2017). It has opened new avenues to study about parasite biology, especially, at places where reasons behind their biological phenomenon were unknown. For instances, absence of maxicircle kinetoplastid DNA and its synthesized oxidative phosphorylation assembly led to adaptation of mechanical transmission by *Trypanosoma evansi* as a deviation from its progenitor, *Trypanosoma brucei* which causes nagana disease and African sleeping sickness and remain confined to African continent (Sanchez et al. 2015). Similarly, mitochondrial sequencing has brought about revolution in molecular taxonomy of parasites (Feagin 2000). It led to the merger of genera *Boophilus* of ixodid tick with the genera *Rhipicephalus* at subspecies level. Another illustration is the revised nomenclature of *Babesia equi* as *Theileria equi* due to greater phylogenetic similarity and positioning under the clade of *Theileria* species

✉ Sachin Kumar
sachin.amroha@gmail.com

✉ Ashok Kumar Chaubey
akcnema@gmail.com

[1] Division of Parasitology, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122, India

[2] Department of Zoology, Choudhary Charan Singh University, Meerut, Uttar Pradesh 250001, India

[3] Department of Veterinary Parasitology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana 125001, India

(Kappmeyer et al. 2012). Further, advancement in bioinformatics with molecular docking softwares such as Autodock, Glide, GOLD, LigandFit, QXP, Flex X, FRED, Dock and several others provides opportunity for advent of new safer and potent molecules for control of parasites (Chaudhary and Mishra 2016). Recently, drug eflorithine has introduced by target based drug identification approach against *Trypanosoma brucei gambiense* by targetting its Ornithine decarboxylase pathway. The Herculean task of sequencing of the complete genomes of protozoa, arthropods and parasitic helminths and their internal organisms (endosymbionts) has taken by several research groups like Sanger institute and the raw and annotated sequence are available in various genomic libraries. Polymerase chain reaction (PCR) and its variants such as multiplex PCR, Race PCR, RAPD, LAMP, nested PCR and qPCR are commonly employed to increase sensitivity and specificity of detection of parasite, identification of parasite species and strains. It can be seen by the fact that TBR and ITS-1 primer based PCR can detect as low as 0.1 ng of the DNA of *T. evansi* in the whole blood (Sharma et al. 2012). Other techniques such as DNA micro-arrays, DNA probes and uniquely designed molecular beacons have greater impact in diagnostic and epidemiological studies of veterinary parasites by providing rapid screening of large number of parasite genotypes. For cloning and sequencing of genes, diagnostics and vaccines production, availability of genomic data library is a great aid for parasitologists. In addition, vaccines such as DNA vaccines and subunit vaccines have shown potential of sustained stimul ation of the host immune system compared with various recombinant protein based vaccines (). There are certain incidences where the recombinant antigen failed to produce immunity, hitherto, native antigen is highly efficacious. It can be seen in case of Barbervax which is world's first subunit vaccine against *Haemonchus contortus* (de Matos et al. 2017). On the other hand, Cystvax, fist cestode vaccine is entirely baed upon recombinant antigen and found highly efficacious against porcine cysticercosis (Thomas et al. 2019). Emergence of drug resistance in parasites is becoming a greater challenge for control and eradication of parasitic disease. The biotechnological tools such as pyrosequencing, allele specific PCR, PCR-RFLP and conventional PCR techniques are also involved in monitoring the development of drug resistance by targeting specific molecular markers (Kumar et al. 2020). In case of pyrethroid resistance in ticks, mutation in sodium channel gene at different location is detected (Kumar et al. 2020). Recently, point mutation in in the *Theileria annulata* cytochrome b gene is associated with buparvaquone treatment failure (Sharifiyazdi et al. 2012). Highly sensitive and specific DNA-based assays targeting resistance-alleles can provide an aid in maintaining the effectiveness of existing antiparasitic drugs and keep the parasitic diseases under control with the current available drugs. In the similar context, bulk of information are available about proteome of protozoan parasites such as *Trypanosoma evansi* based on nanoparticle liquid chromatography adjunct with tandem mass spectrophotometry (Roy et al. 2010). This write up will focus on diagnosis, vaccines, developments in relevant genome projects and reverse vaccinology against parasites of veterinary importance.

Parasite genomics

For diagnostic tools, vaccines and antiparasitic drugs used in controlling the pathogenic parasites, several traditional immunological studies and empirical drug screening had been carried out in veterinary parasitology but the progress achieved in terms of sensitive and specific diagnostic assays, therapeutic drugs and recombinant protein based vaccines remain lesser than expected outcome. In North-east India, there is helminth database called as Northeast India Helminth Parasite Information Database (NEIHPID) that document helminth parasite of that area, their taxonomic character alongwith information on DNA sequences of nuclear, mitochondrial and ribosomal genes marker region and next generation sequencing data for *Paragonimus westermani, Fasciolopsis buski* and *Artyfechinostomum sufrartyfex* (Biswal et al. 2016). Nowadays, there are multidirectional approaches towards research on helminth and protozoan parasites due to the availability of whole genome sequences and improvement in *in vitro* culture techniques. There is huge variation in size of parasites genome from size of the genomes from approximately 10 Mb for *Theileria* to 270 Mb for schistosomes. Amongst kinetoplastids, *Trypanosoma brucei* has a genome size of around 53 Mb and more than 3500 random cDNA ESTs are available. Similar progress has also been made on the *T.cruzi* genome (Ash 1999). The sequencing and annotation of the 366 Mb genome of *Glossina morsitans morsitans* is already completed long back to develop insight into African trypanosomosis (International Glossina Genome Initiative 2014). Recently, genome sequence of *L. cuprina* produced a final draft assembly of 458 Mb which has opened new avenues for determination of drug target and vaccine candidates for control of blow fly infestation in animals (Anstead et al. 2015, 2016). In contrast to other parasites, tick genome is highly complicated.

The *Rhipicephalus microplus* genome is large and complex in structure, representing over 1.8 Gb pairs of DNA which is stored in a database, CattleTickBase (Bellgard et al. 2012). Whole genome projects being conducted on human parasites like *Brugia malayi*, can benefit veterinary parasitologist while working on *Dirofilaria immitis* and other filarial nematodes. Similarly, information on human schistosome, *Leishmania*, *Plasmodium* and *Entamoeba* genome projects, will be useful for research on veterinary parasites such as *Fasciola*, trypanosomatid and other apicomplexan parasites. In 1996, Edna McConnell Clark Foundation has developed OnchoNET which provides series of hypertext links to the lists of available antigens, serum, genomic and cDNA libraries

a listing of researchers currently working on onchocerciasis, which, is the fourth leading cause of blindness worldwide. Genome library serve as the basis for functional analyses of the newly discovered genes. It helps in identifying proteins that are responsible for pathogenesis, host specificity, virulence, involved in protective immune responses and those which are essential components of metabolic processes. Molecular tools such as RNA interference and CRISPR helps in confirmation of functionality of a particular gene. Genome sequencing and understanding of host parasite behavior provide the basis for designing and exploring novel antiparasitic drugs, vaccines, and diagnostic reagents. Several genomics projects on veterinary parasites like *Eimeria* spp., *Schistosoma mansoni, Haemonchus contortus, Toxoplasma gondii* and *Fasciola hepatica*, are either being completed or are on the verge of completion. The expanding genomic and expressed sequence tag (EST) datasets for parasitic flat worms including *Schistosoma* and *Fasciola* have helped in determining the immunogenic epitopes and aid in development of potential drugs and vaccines. As the genomes of helminth, arthropods and protozoa are relatively large in comparison to bacteria and viruses, a common strategy adopted for parasites genomics is gene finding by EST sequencing. Computational cluster analyses of ESTs reveal the relationships between many attributes, such as stage-specific genes regulation and polymorphisms between different strains of a parasite. EST analysis facilitate gene discovery, reduce redundancy and facilitate generation of consensus sequence. There is slow pace of scientific progress associated with evaluation of gene functions in parasites as not much frequently genetic manipulations are carried out on these parasites. Nowadays, functional genomics for parasitic helminths have popularized using RNA interference (RNAi) and transgene expression in several species of nematodes and trematodes for discovering their virulence factors, drug and vaccine targets. RNAi mediated gene silencing has been practiced in few nematodes like *Haemonchus contortus* and *Ascaris suum* and trematodes like *Fasciola hepatica* and *F.gigantica* (Geldhof et al. 2006). Hitherto, no RNAi-related genes like Dicer and Argonaute have been identified in *Plasmodium* and *Leishmania* species so far (McRobert and McConkey 2002; Robinson and Beverley 2003). Apart from macroscopic arthropods, ticks and insects, the mites researchers also have shown interest in gene silencing by RNAi in *Dermanyssus gallinae* and *Sarcoptes scabiei* (Kamau et al. 2013; Fernando et al. 2017).

Recently a workable and functional RNAi pathway in *Fasciola hepatica* was discovered (Mcgonigle et al. 2008; Rinaldi et al. 2008). In newly excysted juvenile of *F. hepatica*, the role of cathepsin-L cysteine proteinase in penetration of its host gut wall during its invasion of liver has been explored with RNAi technique (McGonigle et al. 2008). The RNAi technique has played a key role in determining the role of leucine aminopeptidases (LAPs) in the egg hatching process in the schistosomes (Rinaldi et al. 2009). Through RNAi involving functional genomic study, new opportunities could be explored such as identification of key molecules in the parasite-host interactions, future drug and vaccine targets. Therefore, in near future functional RNAi pathways will be fully explored in *Schistosoma*, *Fasciola*, ticks, protozoa to achieve the ultimate goal of development of a vaccine and therapeutic intervention for these parasites.

Molecular diagnosis

Protozoa

Protozoan parasites of veterinary significance are diagnosed conventionally by microscopy coupled either with Romanovsky stains or immunofluorescence/immunocytochemistry. Conventional techniques include the use of blood smear examination in case of haemo-protozoa (*Theileria*, *Trypanosoma*, *Babesia* etc.), faecal examination in case of intestinal protozoa (*Giardia*, *Eimeria*, *Cryptosporidium)* or tissue smears or intestinal scrapping or sections (*Eimeria*, *Toxoplasma*, *Neospora*) in histozoic protozoan parasites. In several laboratories, chemical test based upon alteration in serum protein and serological test based upon detection of high antibody titres in ELISA or immunofluorescence or complement fixation test (CFT) are employed as main methods for diagnosis of protozoan parasites. However, these techniques suffer from the limitation of poor sensitivity and specificity, high technical skill and large manpower requirement. With advancement in molecular techniques, there are now ranges of PCR and purified antigen-antibody based assays that are routinely used with high sensitivity, specificity and low invasiveness for diagnosis of parasitic disease. The application of microarray has raised the probability of faster detection of molecular marker of protozoan parasites (Akopyants et al. 2004). For *Cryptosporidium*, *Babesia* and *Theileria*, the ribosomal DNA genes have been used in PCR based genotyping and diagnostic techniques (Smith 1998; Morgan and Thompson 1998; Bashiruddin et al. 1999). Highly conserved ribosomal DNA (rDNA) genes are also useful for comparisons between closely related species. The internal transcribed spacer (ITS-1 &2) regions are relatively small, show variability among related species and are flanked on either side by highly conserved segments to which PCR primers can be designed. Individual variations in inter-species length makes the ITS region a useful marker for identification of multiple species within a single sample (Ahmed et al. 2013). In addition, single copy sequences based PCR systems have been developed. It has been found that for the detection of *Trypanosoma brucei* (McNamara et al. 1994), *T. vivax* (Masake et al. 1997) and *T. congolense* (Majiwa et al. 1993), PCR based protocols are well standardized. A repetitive sequence has been well

standardized for sensitive detection of *Toxoplasma* in infected tissue (Johnson et al. 1993) which is conventionally practiced by mice inoculation and hazardous Sabin Feldman dye test. PCR based techniques have been devised to detect parasite specifically from infected tissues in case of *Toxoplasma* and *Neospora* (Muller et al. 1996; Lally et al. 1996) and it has been also demonstrated that it detected as few as one parasite per milligram of muscle tissue or brain (Yamage et al. 1996). Real-time PCR is advancement in PCR techniques for quantifying the amount of original target sequence in the reaction and make it possible for estimation of the number of parasites in a given sample. With the advancement in technology, it is now possible to identify mixed species of parasites simultaneously with the help of Multiplex-PCR. In case of intestinal protozoa, multiplex real-time PCR assay was developed for the simultaneous detection of *E. histolytica, G. intestinalis*, and *Cryptosporidium* spp. in one reaction using species-specific probes, however, high cost limit its use in routine practices (Haque et al. 2007). Similarly, multiplex PCR developed for detection of *Trypanosoma evansi* and *Theileria equi* in equines (Sumbria et al. 2016). Multiplex PCR is also applied for the simultaneous detection of natural infection of theileriosis, babesiosis and trypanosomosis in cattle (Sharma et al. 2014; Kundave et al. 2018). In canines, detection of *Babesia canis vogeli, Babesia gibsoni* and *Ehrlichia canis* could be carried out by multiplex PCR (Jain et al. 2018). Newer techniques such as micro-arrays or molecular beacon's use in parasites of veterinary importance would be a very attractive approach. DNA micro-array technology could play an important role in raising the pace of development of diagnostics in veterinary parasitology.

Helminths

To study parasites and parasitic diseases, there has been a greater development in molecular application over the years. The advancement in application of the polymerase chain reaction (PCR) has greatly influenced the areas of parasite systematics and epidemiology, host-parasite interactions, immunology, and recombinant DNA vaccine development. The increased sensitivity of PCR has wide range of applications involving transformation of this technology not only for diagnostics, but other research areas also. Quantitative PCR is established in the concept that the amount of final PCR product can be used to conclude either the initial number of selected molecules in a given sample (quantitative PCR) or the relative starting levels of target molecules among a number of samples (relative PCR). Changes in transcription levels of the host derived selected genes (expression of cytokine genes) could be studied in interpreting the host response to the infection process.

Helminthic parasitic infection can be diagnosed through several PCR based protocols using genera and species specific primers. This may be followed by restriction fragment length polymorphism (RFLP), PCR-linked single strand conformation polymorphism (SSCP) and PCR linked to hybridization with specific probes as more confirmatory tools for diagnosis of the parasite species, isolates / strains. Also, for discovering the polymorphism in the parasite, random amplified polymorphic DNA (RAPD) based on non-specific primers is a useful tool.

DNA- based assays based on ribosomal DNA sequences such as internal transcribed spacers (ITS-1 and ITS-2), 28 s, 16 s, 18 s and mitochondrial sequences are species specific and provide a DNA region which allows for high sensitivity and specificity for parasitic helminths (Huang et al. 2004). Using this technique with the second ITS spacer (ITS-2), helminth such as *Haemonchus*, *Cooperia*, *Nematodirus*, *Trichostrongylus* and *Ostertagia* genera were differentiated and detected based on single egg (Schnieder et al. 2010). Different strains within a parasitic species have been differentiated successfully based on DNA-based assays (Zarlenga et al. 1999) and to evaluate biological differences between them. Using ITS-PCR, differences in pre-patent periods between different isolates of *Oesophagostomum* was also studied (Talvik et al. 1997). The strain/genotype identification in *Echinococcus* for determining host specificity has been made possible with PCR and sequencing techniques. In fact, PCR has become a crucial molecular assay in the studies on helminth parasites of veterinary importance in their genotyping, strain, diagnosis, identification and providing DNA for promoting recombinant antigens for vaccine development.

Vaccine development

Protozoa

Live attenuated vaccines are available in case of *Babesia bovis* (Callow et al. 1997), *Eimeria* spp. (Shirley and Bedrnik 1997), *Toxoplasma* (Buxton et al. 1991) and *Theileria annulata* (Pipano 1995), however, they suffer from certain disadvantages such as possibility of contamination with other pathogens, need of a cold chains for maintenance of efficacy, tedious production, reversion to pathogenic forms and low shelf life. With the advancement in molecular biology, there are certain sub-unit vaccines in existence for *Leishmania, Plasmodium* and *Eimeria* species.

While our knowledge of protective immune mechanisms involved in *B. bigemina* and *B. bovis* infections is not complete, it is clear that antibody plays a significant role (Mahoney 1986) and there is greater proof for a notable role for CD4 + T-cells and activated macrophages (Brown and Palmer 1999). Systematic testing of fractionated *B. bovis* merozoite proteins have resulted in identification of several antigens that conferred a level of protection to vaccinated animals (Wright et al. 1992). Under field conditions, combinations of

these antigens, tested gave notable protection as well as protection levels close to those provided by the live vaccine. A set of antigens have been identified using CD4 + T-cell screening (Jasmer et al. 1992; Hines et al. 1992; Brown et al. 1996) that include a set of spherical body antigens (SBPs) and two major merozoite surface antigens that are secreted by the parasite and located on the cytoplasmic face of the red blood cell (Dowling et al. 1996). T-cell epitopes mapped for rhoptry protein RAP-1 are considered as a major candidate antigen for inclusion a sub-unit vaccine (Brown et al. 1996, 1998). RAP-1 proteins in *B. bigemina* immunization manifest a protective effect on challenge, however, only reduction in parasitaemia and no complete protection occurs (Brown et al. 1998; McElwain et al. 1991). Research on the development of a sub-unit vaccine against *T. annulata* and *Theileria parva* has concentrated on the clarification of the mechanisms of immunity and the isolation of surface antigens and their characterization as they are found to be predominant in host cell invasion. Moreover, the protective immune response to *T. parva* is because of a major role for cytotoxic T-cells (McKeever et al. 1994) with parasite peptides presented on MHC Class I molecules of infected lymphocytes acting as the target that mediates their lysis (McKeever et al. 1999). The protective response to *T. annulata* involves a complex interplay between activated macrophages, NK cells, and cytotoxic T-cells (Preston et al. 1999; Boulter and Hall 2000). Moreover, the humoral response has a little role in intracellular parasites infected animals. The laboratory based immunization trails of major sporozoite surface antigen (SPAG) from both species has been used as recombinant antigen (Boulter et al. 1995, 1998; Morrison and McKeever 1998), with a covering a protective response up to 70% in *T. parva* (Musoke et al. 1992) and notable decrease in a number of disease parameters including parasitaemia with *T. annulata* (Boulter and Hall 2000). The recombinant SPAG-1 protein delivered with RWL showed better protection against challenge than when delivered with ISCOMS (Boulter et al. 1998, 1999). d'Oliveira et al. (1997) expressed these proteins in *E.coli* using gene fragments lacking both hydrophobic domains to assess the potential of these molecules for use in diagnosis and as components in a multi-unit recombinant vaccine.

Sequence analysis of the many alleles of *Ta*ms has shown that they encode the highly polymorphic molecules, particularly within a region that contains a number of putative N-linked glycosylation sites (Katzer et al. 1998). It is postulated that this level of antigenic diversity may indicate selection of variable glycosylation sites or amino acid epitopes in order to evade the host immune response (Shiels et al. 1995). For a sub unit vaccine to be successful; it requires the inclusion of antigens that stimulate cellular responses to the schizont infected lymphocytes. Approaches to the identification of such molecules are being developed and include (*T. parva*) the elution of peptides from MHC Class I molecules to evaluate their ability to sensitize target cells to cytotoxic T-cell killing (McKeever et al. 1999). Along with identification of active molecules, the problem of antigen delivery needs to be checked.

Arthropods of Veterinary and Medical Importance

Arthropods such as ticks, mites, flies, bugs and lice are responsible for both direct and indirect losses to the livestock industry. Next to the chemotherapy using ecto-parasiticidal and repellant agent, major thrust of the researchers is to establish a sustainable immunological control of arthropods by development of suitable vaccines. In this direction, major work in veterinary parasitology is carried out on the control of one host tick, *Rhipicephalus microplus*. Vaccine against the tick *Rhipicephalus microplus* is based on successful recombinant antigens commercialised in market (Tick GARD Plus in Australia and GAVAC Plus in Cuba). Later, Tick GARD Plus was withdrawn by Australian government due to low economic return; however, it is still continued in Cuba. This vaccine contains concealed immunogenic mid gut antigens (Bm86, Bm91) and vaccination leads to decrease in the number of engorged females, increased red ticks due to bursting of mid gut and the reduction up to 90% in the number of larvae per generation (Willadsen and Jongejan 1999). Initially, studies on tick immunological control address mostly single-antigen vaccines. However, so far no single-antigen vaccine has afforded appropriate protection against all *R. microplus* populations, therefore, new modified vaccines were launched but they were also proven ineffective in other countries due to strain variation. In this context, to enhance vaccine efficacy, multi-antigen cocktails are evolving as a novel methodology in tick control strategies. Moreover, to have a protective immune responses against heterologous tick challenges, coomon situation in tropical countries like India, identification and development of a universal anti-tick vaccine such as based on salivary antigen like aquaporin or structural component of body like cystatin and any other combination involving one or more common tick antigens and would be economically and technically attractive. Also, the finding of an antigenic protein conserved between ticks and mosquitoes (Canales et al. 2009; Prudencio et al. 2010) have increased the possibility of a pan-arthropod vaccine. The development of a universal vaccine could depend on highly conserved tick proteins with narrow and reasonable antigenic variations, which are capable of involving in a protective cross-reactive immunity against different tick species (Parizi et al. 2012a, b).

Fly menace is another major problem globally, especially, in tropical and subtropical part of world. Few researchers are attracted towards immunological control for mitigating the losses due to flies in livestock industry. There is advancement observed in vaccine development against *Culicoides, Lucilia* and *Hypoderma* flies. Insect-bite hypersensitivity (IBH) in horses is manifested by chronic relapsing seasonal allergic

dermatitis and is mainly caused by the biting of *Culicoides* flies. IBH is a IgE-dependent type I allergy, with a strong involvement of type IVb allergic hypersensitivity reactions in dermatitis resulting into recruitment of eosinophils into the allergic site. Type IVb allergy is associated with IL-5 producing TH2 cells, eosinophilia, and eosinophil accumulation in perivascular clusters in deeper parts of the dermis. Eosinophil development in the bone marrow and release and activation in blood circulation is mediated by IL-5. Mice either vaccinated against murine IL-5 or IL-5–deficient (knockout) mice showed strongly reduced levels of blood eosinophils and eosinophil-mediated inflammation. Furthermore, anti–IL-5 mAb, significantly reduced circulating eosinophil. Gabriel and coworkers (Fettelschoss-Gabriel et al. 2018) targetted IL-5 for mitigating the allergic reaction in equines due to bite of Culicoides flies. Targeting IL-5 with mAbs is not a realistic approach in equines due to large weight and corresponding high dosages. Recently, a novel VLP platform has been developed based on the cucumber mosaic virus containing the tetanus toxoid universal T-cell epitope tt830-843 (CMVTT) to enhance TH cell–dependent IgG responses for antigens displayed on its surface. Accordingly, a vaccine consisting of equine IL-5 (eIL-5) chemically linked to CMVTT-VLPs has found to induces potent anti–eIL-5 antibody responses, which improve symptoms of IBH in horses. A second vaccine using the same CMVTT VLP backbone but targeting equine IL-31 was tested by the same group of researchers. IL-31 is a Th2 cell cytokine that interacts with the nervous system via the IL-31 receptor expressed by dorsal root ganglia cells in the skin and triggers the allergic pruritus (Sonkoly et al. 2006; Mizuno et al. 2009). In IBH, IL-31 was found to be exclusively expressed in punch biopsies of IBH-affected skin lesions (Olomski et al. 2020). Further, studies including larger patient cohorts and combining both IL-5 and IL-31 vaccines may elucidate significant benefit of the vaccines in IBH-affected horses (Jonsdottir et al. 2019).

Peritrophic membrane (PM) lines the gut of many arthropods and separates ingested food from the gut epithelium. PM restrict the penetration of ingested immune effector components f host and in other ways, it can serve as a target for immunological attack of host. Sheep vaccinated with extracts of PM from larvae of the sheep blowfly, *Lucilia cuprina*, slowed the growth of *L. cuprina* larvae (Eisemann and Binnington 1994). In a another study, a mucin-like glycoprotein, peritrophin-55, isolated and purified from *L. cuprina* larvae inhibited larval growth by 51–66% when larvae fed on sera from the vaccinated sheep (Tellam et al. 2003). The situation with another myiasis fly, *C. bezziana* is also similar on vaccination with Cb15, Cb42 and Cb48 antigens. Vaccination of sheep leads to dramatic reduction in the weight of larvae recovered from both *in vitro* and *in vivo* assay systems. Fractionation, characterisation and expression of a number of peritrophic membrane proteins have proven to be ineffective so far (Sukarsih Partoutomo et al. 2000).

Helminths

Great successes had been achieved against bacterial and viral pathogens related to vaccine, but little success has been achieved against eukaryotic parasites due to similar cellular machinery. There are fewer number of successful anti-parasite helminth vaccines developed, however, until recently all were based on the use of living, attenuated parasites. Examples are the vaccines against tropical theileriosis (Pipano 1995), babesiosis (Callow et al. 1997), and lungworm in cattle (Jarret et al. 1960), sheep and goat (Dhar and Sharma 1981), ovine nodular worm (Dhar and Sharma 1980), canine hookworm, *Ancyclostoma caninum* vaccine (Miller 1978) and coccidiosis in chickens (Shirley and Badrnik 1997). With the beginning of molecular era, recombinant antigen based vaccine comes into existence. World first cestode vaccine, Cysvax is commercialized by Indian immunological to curtail the case of porcine cysticercosis. Similarly, Barbervax vaccine was launched for the control of *Haemonchus contortus* in sheep, hitherto, it is a native antigen based vaccine and large scale production rely upon slaughter house availability. These vaccines antigens were identified, confirmed and produced using recombinant DNA approaches, after doing immunochemical analysis with the help of molecular tools. The recombinant vaccines against cysticercosis and hydatidosis in sheep and cattle have long been developed, but away from commercialization due to poor market response. Moreover, three highly immunogenic oncosphere antigens have been identified from *Taenia ovis* (To 45W, To16.17 and To18) and expressed recombinantly, and it produced 73–99% protection in sheep challenged with *T. ovis* (Harrison et al. 1996). Similar, high levels of protection have been found against *T. saginata* (Tsa 9 and Tsa 18) in cattle (99%) with recombinant oncosphere antigens (Lightowlers et al. 1996a, b) have shown protection against *T. solium* (To 45W, To 16.17 and To 18 derived from *T. ovis*) in pigs (93%) (Plancarte et al. 1999) and *E. granulosus* (EG 95) in sheep (97%) (Lightowlers et al. 1996a, b). Further, glutathione-S-transferase (GST), fatty acid binding protein (FABP) and cathepsin L1 and L2 and hafve been explored in vaccination studies against *F. hepatica* and *F. gigantica* in sheep and cattle. However, moderate level of protection against worm numbers has been found. A potential vaccine candidate, *F. hepatica* leucine aminopeptidase (LAP) has shown significant protective efficacy ≥ 85% against a challenge infection by *F. hepatica* in sheep (Maggioli et al. 2011). Commercialization of these vaccines could lead to a breakthrough in veterinary parasitology.

Since the commercialization of a recombinant vaccine against the Australian cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* (Willadsen et al. 1995), substantial work has been done on "concealed antigens" in nematode parasites. Medium to strong levels of protection have been recorded in sheep vaccinated with *H. contortus* gut aminopeptidases 'H11'

(Smith et al. 1997). Other antigen, H-gal-GP, a glycoprotein complex containing predominantly digestive proteases, is a promising vaccine molecule (Ekoja and Smith 2010). Other proteins of *H. contortus* which are of interest for vaccine development are an extensive family of cathepsin B-like cysteine proteases (Rehman and Jasmer 1999), glutamate dehydrogenase (Skuce et al. 1999), Cu / Zn superoxide dismutases (Liddell and Knox 1998), etc. As the whole genome sequencing of pathogenic helminths such as *H. contortus* (370 Mb) has been completed (Laing et al. 2013), this will likely increase the process of development of highly effective anti-nematode vaccines.

Reverse vaccinology

The field of reverse vaccinology developed is an outcome of the genome sequence revolution. Vaccine candidates for control of theileriosis, leishmaniasis, malaria, schistosomiasis, anaplasmosis and the cattle tick have been identified using reverse vaccinology approaches (Lew-tabor and Valle 2016). Reverse vaccinology (RV) involves use of bioinformatics analysis of genome sequence data along with the laboratory screening to identify new vaccine candidate (Rappuoli 2000). It involves new approach of not involving a pathogen to be cultivated for discovery of vaccine. For vaccine design, computers are used and no live organism is involved. It provides all the protein antigens that a pathogen can express at any time by genome sequence analysis. This approach was used in small genomes pathogens and resulted in the first successful vaccine produced against meningititis and sepsis causing bacteria (type B *Neisseria meningitidis*) in children and young (Adu-Bobie et al. 2003; Rappuoli 2000). Reverse vaccinology approaches for eukaryotes have also recently been used but that describes the detail study of vaccine candidate identification and not the approach applied (Schubert-Unkmeir and Christodoulides 2013). For many parasites species including *Schistosoma* (Wang et al. 2016), *Cryptosporidium* (Manque et al. 2011), *Toxoplasma* (Sette and Rappuoli 2010), *Giardia* and *Trichomonas* (Aslett et al. 2009) many databases 'omic' and EST have evolved with the arrival of more genome sequences. In an *in silico* study of *Neospora caninum* using RV approach, several potential vaccine candidates are shortlisted and ranked (Goodswen et al. 2017). For the parasites, RV approach had not yet produced any commercialised products. Protozoan genome based RV approach for the genus *Leishmania* was developed (Rezende et al. 2012). For parasite (protozoa) and veterinary vaccines such as coccidiosis, the databases and *in silico* tools for vaccine design have kept evolving for example, VIOLIN—Vaccine Investigation and Online Information Network. (He et al. 2013; He and Xiang 2013). Recently, RV approach was used for identification and characterization of *Ctenocephalides felis* protective antigens for the control of cat flea infestations (Contreras et al. 2018).

Conclusions

Genome information on arthropods, protozoa and helminths opens up new understanding on parasite biology and advanced approaches for improving diagnosis and control measures. DNA micro-arrays can help in exploring the new knowledge of parasite genomes. Host-parasite interactions and parasite immune evasion mechanisms understanding will lead to the development of successful vaccines. Using knowledge and tools of molecular biology and bioinformatics in teaching, research, and clinical work will accelerate the expansion of vaccines, diagnostics and newer anti-parasitic drugs for controlling the risk of parasitism in our livestock. The multivalent molecules developed for bacterial vaccines using reverse vaccinology approaches can act as key for future parasite vaccine development.

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