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Advances Techniques in The diagnosis of Helminthes of Livestock

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INFECTION of the gastrointestinal tract (GIT) with helminths includes the three main groups: nematodes, cestodes and trematodes. They are detected by using conventional methods either by direct smear method or floatation /sedimentation technique which are laborious, time consuming and exhibited low sensitivity. Currently, recent progress in new diagnostic tools has opened new avenues in helminths detection. The immunological techniques which include enzyme linked immunosorbent assay (ELISA) and its modifications were appropriate for such diagnosis. They showed high sensitivity and specificity for such diagnosis. In addition, progress in molecular technique provide the potential for more reliable and efficient methods for diagnosis of hhelminthes infection. Molecular methods such as PCR (the polymerase chain reaction) , RLB (reverse line blotting), RT-PCR (real time- PCR), LAMP (loop-mediated isothermal amplification), and RFLP (restriction fragment length polymorphism) can be used as specific and sensitive tools for accurate detection of parasites DNA. PCR-based methods can be joined with RFLP or nested PCR for parasites genotypic. These combined methods can give different technique for the specific pathogen detection in stool. As well as, detection of low number of helminth parasites in stool samples by PCR is considered useful due to the higher detection sensitivity of PCR comparing to light microscopy. Recently, LAMP technique is helpful in detection of many parasitic agents and it is considered a golden tool for detection of helminths. Also, RLB method is a suitable diagnostic tool to define the characters of species in mixed infection.

Keywords: Diagnosis; helminths; livestock; ELIZA; PCR.

Introduction

Helminthic infestations are among the most common infections in man and livestock. These infestations produce a global burden of disease and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia which are more often physically impair their hosts than killing them [1, 2]. Diseases caused by helminths in humans and their livestock persist to be a major constraint, particularly in the subtropics and tropics [3].

Infection of the GIT with helminths includes the three main groups: nematodes, cestodes and trematodes. Many species of nematodes and cestodes cause parasitic gastritis and enteritis in livestock. The most important of these are *Haemonchus contortus* , *Trichostrongylus axei*, *Ostertagia circumcincta*, intestinal species of *Trichostrongylus*, and *Strongyloides* . Trematode infection for GI tract such as Schistosoma and *Fasciola spp.* leading to block and hepatic damage [4]**.** A wide range of GI nematode parasites are responsible for significant clinical and veterinary problems worldwide, and have been identified in both humans and animals [5].

Some parasites such as *H. contortus* suck large volumes of blood and cause clinical anemia. Their

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hematophagous nature causes degeneration of epithelial cells of GI tract and damage in mucosa which has been responsible for greater mortality of animals [6]**.** Also, some hhelminthes like *Fasciola* spp. have been known to cause organ damage due to either inflammatory reactions or mechanical effect . This will lead to severe morbid and reduction in productive and reproductive performances.

In Egypt, the prevalence of GIT parasites in 240 sheep was conducted from the zoo garden and Sinai district. The overall prevalence of infections in Sinai and zoo garden were 27.5%; 10.0% and 6.7% with nematodes; *Fasciola* spp. and coccidiosis respectively [7]**.** In addition Sultan et al. [8] investigated the prevalence, and public health importance of the GI parasites of sheep from Nile-Delta. The prevalence of GI parasites in a total of 224 individual sheep was 50%: Protozoa (29.02%) and helminths (37.05%). The prevalence of helminths infection was by *Strongyle* group (19.21%), *Paramphistomes* (9.38%), *Strongyloides papillosus* (4.02%), *Trichuris* spp. (2.68%), *Moniezia* spp. (0.89%) and *Nematodirus* spp. (0.45%).

For a long time, microscope has been considered the only tool available for the detection of helminths through tissue samples and feces. However, sample making ready for direct observation is labor keen, time-consuming, and depends on qualified laboratory technicians. Indeed, all major intestinal hhelminthes infection are still entirely contingent on microscope for diagnosis [9]. Many parasite infections are confirmed by the use of other methods of diagnosis including serology-based assays and molecular-based assays in conjunction to microscopy [10]**.** Most of GI helminths are transmitted orally, but they differ in their definitive and intermediate hosts. They are detected by using conventional methods either by direct smear method and floatation /sedimentation technique which are laborious, time consuming and exhibited low sensitivity [11]**.** In addition, some nematode eggs did not float in NaCl [12] **.**Currently, recent progress in diagnostic tools have achieved new avenues for improvement in helminths diagnosis. The immunological techniques which include ELISA and its modifications were appropriate for such diagnosis. ELISA have previously been reported for diagnosis of *H. contortus* [13] and *T. circumcincta* [14] in sheep and *Ostertagia ostertagi* in cattle, [15] it showed 99% sensitivity of diagnosis for such infections. Also, latex agglutination assay showed 100% sensitivity by using *H. contorus* crude antigen for diagnosis of sheep haemonchiosis [16]. In addition, indirect ELISA and Western blotting (WB) in the diagnosis of sheep haemonchosis were applied by Sultan et al. [17]. However, infection of cattle with tapeworm, *T. saginata,* or *Cysticercus bovis,* also known as bovine cysticercosis, occurs worldwide [18]**.** Ogunremi and Benjamin [19] applied new trial for identification of *T. saginata* metacestodes in bovine lesions by using immune-histochemical stain complex. The most important GI trematodes that have zoonotic importance are *F. gigantica* and *F. hepatica*. They infect human and wide range of livestock as cattle, buffaloes and sheep and cause fasciolosis. Indirect ELISA proved 92% and 94.4% specificity and sensitivity, respectively in the diagnosis of cattle and sheep fasciolosis [20, 21]**.** In the last decade, several molecular tests have been developed to detect parasites in which their specificity and sensitivity have gradually been increased. Molecular methods such as the polymerase chain reaction (PCR), reverse line blotting (RLB), real time- PCR (RT-PCR), loop-mediated isothermal amplification (LAMP), and restriction fragment length polymorphism (RFLP) can be used as specific tools and sensitive for parasites DNA detection of [22, 23]. PCR-based techniques have a great role in the revolution and development of many areas of researches because only small amounts of material can be used for *in vitro* enzymatic amplification of DNA. This point is specifically important to parasitologist as it is commonly not possible to isolate large amount of parasite materials at their life cycle stages for typical analysis [24]. The cyathostomins helminthes (small strongyle) are considered the most important and common GI helminths infecting horses in livestock [25]. The eggs of cyathostomins origin were determined by larval cultures. The differentiation of cyathostomins group to species or genus level doesn't determined by these culture. So molecular techniques have been applied for detection cyathostomins in faecal samples. These include PCR-ELISA and RLB assays [26, 27]**.** Furthermore, Learmount et al. [28] tested the validation of a RT- PCR method for diagnosis of *T. circumcincta* and *H. contortus* in sheep. LAMP technique is helpful in detection of many parasitic agents such as *Taenia*, *Schistosoma* and *Fasciola spp* [29, 30]. LAMP method is more sensitive than PCR in differential recognition of *Taenia* in stool samples. So, it is a golden tool for

detection of taeniasis [22]. In addition, there are many studies have been concerned with markers (mitochondrial DNA- autosomal markers) such as microsatellites. These microsatellites have been applied only to a nematode species, like Trichostrongyloid [31]. The goal of this review is to highlight for the recent diagnostic technologies in GI helminths that affecting farm animals.

Immunological diagnostic techniques

The immunologist researcher efforts are directed to develop easy, fast and less expensive methods in addition to high specific antigens and antibodies that can be used in immunological techniques. These can be useful in serology that in situations where samples are unavailable. The diagnosis which based on the serological tools can be divided into two categories: detection of antibody assays and detection of antigen assays. The serological tools include ELISA assay and other modifications assays like the Western blotting (WB), direct or indirect immunofluorescent antibody hemagglutination, rapid diagnostic tests , and complement fixation test [10]**.** ELISA is considered the suitable for evaluation of antibody titer and can also be successfully employed for the quantitative assessment of an antigen in a sample, often devised in convenient easy to use kit formats.

ELISA have previously been reported for diagnosis of *H. contortus* [13] and *T. circumcincta* [14] in sheep and *Ostertagia ostertagi* in cattle, [15] it showed 99% sensitivity of diagnosis for such infections. Moreover, it allows sero-epidemiological studies and detection of infection in massive breeding of livestock [32, 33]. Cyathostomins (small strongyles) are considered the most important GI helminths in horses worldwide. These include *Anoplocephala perfoliata,* S. *vulgaris*, and *Parascaris equorum*. All have been related with weight loss, poor growth, and clinical symptoms [34-36]. Dowdall et al. [37] showed that a protein named cyathostomin gut-associated larval antigen-1 have shown a promising diagnostic potential for detection of encysted small strongyles. This protein was only expressed in the larval stages and specific for cyathostomins species.

Indirect ELISA and WB in the diagnosis of sheep haemonchosis were applied by Sultan et al. [17]. They used crude *Haemonchus* adult antigen which proved 87.5% sensitivity and 75%, a specificity. *H. contortus* somatic antigen was purified using gel filtration column chromatography and three purified fraction were obtained. In a vaccination trial these bands were success in the reduction in fecal egg counts and worm burden in experimentally infected lambs. It might be utilized in diagnosis of haemonchosis [38]. Furthermore, Kandil et al. [39] used the immune-reactive protein profile of different prepared *H. contortus* antigens and the indirect-ELISA test for serological diagnosis of haemonchosis. Larval antigen is the prospective antigen for such serological diagnosis. Immuno-dominant reactive band at 57 kDa were liable for high specificity and precision of positive predictive value of this antigen. In addition, larval and excretory secretory antigens showed the highest apparent prevalence values (92 and 75%, respectively). Recently, the indirect ELISA was used for investigation of [40] the early changes in Th1 and Th2 cytokines for diagnosis of strongyle infection in equines with estimation of diagnostic accuracy values; percentage of immunoglobulin G, sensitivity, specificity, positive predictive value, and negative predictive value of different prepared strongyles antigens CSS (crude somatic *S. vulgaris*), ESS (excretory secretory *S. vulgaris*), CSC (crude somatic *Cyathostomins*) and ESC (excretory secretory *Cyathostomins*). Lowest 37.81% and highest 437.04% IgG in low and high egg-shedder groups when using CSS and ESC antigens, respectively. Cattle are considered the intermediate hosts of *T. saginata*, the larval form (metacestode) characterized by the localization in the muscles of infected animals [41]. A more effective method of identifying *T. saginata* metacestodes in bovine lesions has been applied by Ogunremi and Benjamin [19].

They used a complex stain (avidin–biotin and monoclonal antibody to *T. saginata* with diaminobenzidine chromagen and hematoxylin counter stain) against a secretory product of *T. saginata* metacestodes. Degenerated cysts and viable were identifiable after immunohistochemical staining and could be differentiated from other cysts like *Actinobacillus, Sarcocystis,* or normal bovine structures.

Echinococcus species is the most important tape-worms and measured about (3-6 mm long). It is live in the small intestine of carnivorous definitive hosts, such as wolves and dogs, while, cyst stages (echinococcal cyst) are found in intermediate hosts, such as cattle, sheep, goats, camels, pigs, and horses and are called cyctic echinococcosis (CE). Due to cross-reactivity with

other species of taeniid cestodes [42] or to other helminths [43], accurate serological diagnosis of CE infection is difficult. In sheep, which is considered as the main intermediate host of *E. granulosus* in most countries of endemic infection [36], antibodies can be detected at 4 to 6 week post infection [44] and persist for at least 4 years [45] . Indirect-ELISA and WB techniques were used to recognize a specific protein of hydatid cyst fluid (HCF) antigen by CE-infected sheep sera. Three antigens; a crude protoscolex preparation, a recombinant EG95 oncosphere protein and purified 8kDa hydatid cyst fluid protein (8kDa) were adopted for diagnosis of sheep CE, the ELISA test showed highest diagnostic sensitivity with protoscolex antigen followed by 8kDa HCF protein then protein of recombinant EG95 oncosphere . They revealed that the diagnostic specificities were ranged from 96 to 99 % and the immunogenic reactive bands in the crude protoscolex antigen preparation were ranged from 70 to 150 kDa [46, 47]. Furthermore, Jeyathilakan *et al*. [48] demonstrated that the WB assay was the most accurate test (99%) for the detection of CE in sheep by using 8 kDa hydatid cyst fluid antigen.

ELISA and Dot- ELISA were used [49] for diagnosis and detection of circulating antigen of cystic echinococcosis in buffaloes. The specificity and sensitivity were determined as 92 and 89 % for ELISA, whereas those of Dot- ELISA were determined as 96 and 94 % respectively. ELISA was adopted to detect the total specific *E. granulosus* IgG and IgG subclasses antibodies of human CE by using hydatid cyst fluid antigen (HCF) obtained from camel. It showed high sensitivity for such diagnosis [50]. In addition, Ramadan et al. [51] applied enzyme linked immune electro transfer blot assay (EITB) for diagnosis of 47 pulmonary CE cases by adopting human and camel HCF antigens. They found that a six antigens with molecular weights 5, 7, 20, 28, 35 and 127 kDa exhibited diagnostic efficacy. They were strongly recognized by all CE patient sera and the camel HCF antigen proved 100% sensitivity and specificity**.** Moreover, HCF partially purified antigen of camel origin recorded 100% sensitivity in serodiagnosis of hydatidosis in camel and donkey using ELISA, and the specificity was 97.6 and 95.9% respectively [52]**.** Furthermore, HCF crude antigen of camel and sheep origin can be used in diagnosis human hydatidosis using immunoblotting analysis (IB), and recognized 11 major protein fractions [53]. Dipstick assay has been tested on sera from twenty six CE patients and sera from thirty five infected cases with other parasite. By using camel hydatid cyst fluid as antigen the test exhibited 100% sensitivity and 91% specificity [54].The ELISA kits (dipstick assay) is highly easy to perform within 15min with a visually interpretable result. In addition to its specificity and sensitivity, it could be an alternative for use in clinical laboratories lacking specialized equipment or the technological expertise needed for western blotting (WB) or ELISA [54].

Also, Hassanain et al. [55] detected the immune reactive bands of molecular weights ranged from 25- 125 KDa in HCF and protoscoleces crude antigens from camel and sheep using IB. Three main immune reactive bands were observed at 92, 52.2 and 35.7 kDa. These bands may help in diagnosis of human CE. However, the reliability of indirect ELISA in detecting bovine cysticercosis was achieved [56] by using two different crude antigens (*T. saginata* adult worms from human patients and *T. saginata* cysticerci from cattle). 61.76% samples were positive by ELISA with *T. saginata* antigens while 29.4% with bovine cysticercosis antigen.

The indirect ELISA was used for detecting antibody against *F. hepatica* in serum of naturally infected cattle and experimentally infected calves. The test can detect the antibodies at 2-4 weeks post infection in experimentally infected animals, while it proved 98 % and 96% sensitivity and specificity, respectively in detection of antibodies in natural infected cattle [57]. Mandal et al. [58] isolated and purified the glutathione S-transferase from *F. gigantica* extract and successfully utilized by ELISA in early detection of fasciolosis at two week post infection in large ruminant**.** Also, Anuracpreeda et al. [59] used sandwich ELISA based on monoclonal antibodies 3A3 and biotinylated rabbit anti-recombinant fatty acid binding protein (FABP) antibody for detecting of *F. gigantica* circulating FABP in experimental infected mice and natural infected cattle. This assay succeeded in the detection of *F. gigantica* infection at one day post infection in experimentally infected mice and natural infected cattle and recorded 96, 100, 99% sensitivity, specificity and accuracy, respectively. However, Shalaby et al. [60] applied ELISA technique to determine the specificity of antigens for *F. gigantica*. They were tested three antigens: ES (excretory–secretory), egg, and coproantigen antigens. They observed

an intensive cross reaction between egg and ES antigens even when there was no cross-reaction with coproantigen.

Molecular diagnostic techniques

Nowadays, molecular techniques facilitate the diagnosis and identification of the parasites that were previously hard to be diagnosed by conventional techniques. Consequently, treatment can be easily applied before initiating large damage to the infected population. PCR-based methods can be joined with other techniques such as RFLP or nested-PCR for parasites genotypic. In addition, these combined methods can give a specific pathogens detection in stool sample. As well as, detection of low number of helminths parasite in stool samples by PCR is considered useful due to the higher detection sensitivity of PCR comparing to light microscope [61]. The advantage of PCR-based technologies is the detection of some parasites with high specificity and sensitivity. The major disadvantage is the necessary of prior information about the target sequence to produce the primers that will permit its selective amplification. Also, PCR are very time-consuming and cannot give quantitative data. Advanced PCR-based methodology was improved which is the quantitative real-time PCR (RT-PCR) [61]. It considered a sensitive method for detection and identifying protozoa in human feces samples [61]**.** Unlike standard PCR. It is characterized by avoiding using gel-electrophoresis method. This technique therefore usually provides more rapid results and / or uses fewer reactants [62]. In addition, random amplified polymorphic DNA (RAPD) technique is a type of PCR. It characterized by the random amplification of DNA segments. It has been broadly applied for characterization of parasite strains in epidemiological studies [63]**.** RAPD is the method used to describe strains and determine the genetic structure of microorganisms [64]**.** It shows high efficiency of amplification profiles such as studies on parasitic nematodes of humans and livestock. Also, it has been applied to map genes for differentiation of species [65]. RAPD enabled the differentiation of endemic Wuchereria strains in Asia [66]. Sharbatkhori et al. [67] used RAPD to differentiate 112 isolates of *E. granulosus* in ruminants. In addition, Bobes et al. [68] determined the genetic variability of *T. solium* in some locations of USA. RAPD is uncomplicated, rapid, and low-priced test that does not require previously information about the DNA sequence or DNA hybridization [66, 69]**.** Studying the genetic structure of organism by RAPD assay is useful because it detects polymorphisms in the noncoding regions of the genome [64]**.**

LAMP (Loop-mediated isothermal amplification) is an isothermal nucleic acid amplification technique. It does not require a thermal cycler like conventional PCR, and it is carried out at a constant temperature [29]. This technique could be used to amplify limited copies number of target DNA in less than one hour [70]. For example, it can be used to produce rapidly a twenty microgram of DNA from twenty five microliter reaction mixture in 1 h under isothermal conditions with great specificity and sensitivity [30]**.** LAMP technique is helpful in detection of many parasitic agents such as *Taenia*, *Schistosoma* and *Fasciola* spp [29, 30, 70]. LAMP method (88%) is more sensitive than PCR (37%) in differential recognition of *Taenia* in stool samples. So, it is considered a golden tool for detection of taeniasis [22]. In addition, it has a prospective clinical application in differentiation of *Fasciola* spp. in endemic areas. It was ten times more sensitive than conventional PCR in amplification *Fasciola* spp. DNA in stool samples and in mollusks (intermediate hosts) [71]. LAMP is simple and applicable tool for small laboratories. It only needs simple devices as water bath or heat block for amplification of target DNA. There is no need for long cycles and varying temperatures of thermal cyclers. Thus, LAMP method seems to be a promising tool where it is more specific and faster in time than conventional PCR [70].

Restriction fragment length polymorphism (RFLP) was used to detect the variations in homologous DNA sequences [72]. It is commonly used for genotypes of parasites and diagnosis of species [63]. Differential diagnosis of dog hook worms by RFLP was applied [73, 74]**.** The RFLP is appropriate for environmental samples because it can detect multiple genotypes in the same sample. It is an important tool in genome mapping and localization of genes for genetic disorders and determination of risk for disease [63].

Microsatellites is known as short DNA sequences (about 300bp) which are composed of tandem repeats of 1 -6 nucleotides with about 100 repeats [75]. Microsatellites are abundant in genomic eukaryot and can rapidly mutate by losing or gaining repeat units [14]**.** In parasitology, microsatellites have been used to describe some parasites of both humans and animals. There

are many studies have been concerned with mitochondrial DNA markers, and microsatellites autosomal markers have been applied only to a nematode species, like *Trichostrongyloid* [31]**.** Microsatellites have wide diversity of applications because they display frequent polymorphism, high reproducibility, co-dominant inheritance and high resolution, need easy typing methods, and can be observed by PCR [75]. Due to the high number of microsatellites, these genetic markers have low popularity which cause technical difficulties in isolating parasites by PCR [14, 31].

Diagnosis of Nematodes based on molecular techniques

Traditional techniques for diagnosis of the helminths infection in sheep need exhausted laboratory extraction, examination of eggs by culture and microscope. Recent molecular technique provides the potency for more reliable and efficient methods. A combined molecular approach and microscopic examination of strongylid infection in sheep were applied [76]. This method is depending on the isolation of nematode eggs from faecal samples using flotation technique. Specific and semi quantitative genomic DNA amplification from of *T.circumcincta*, *H. contortus*,., *Cooperia oncophora*, *Trichostrongylus* spp,*, O. venulosum* and *Chabertia ovina Oesophagostomum columbianum*, are achieved [76]. This method showed that there was a correlation between numbers of egg per gram of faeces and cycle threshold values in the PCR, so permitting the semi-quantitation of parasite DNA in faeces. This combined method provides a useful tool for diagnosis and epidemiological surveys. In addition, Learmount et al. [28] tested the validation of a RT- PCR method for diagnosis of *T. circumcincta* and *H. contortus* in sheep. A strong correlation has been found between the numbers of eggs determined by the traditional and the molecular methods.

The eggs of cyathostomins in faecal samples were determined by larval cultures but the differentiation of cyathostomins group to species or genus level doesn't determined by this culture. So detection of cyathostomins in faecal samples have been applied by molecular techniques. This includes PCR-ELISA and RLB assays**.** Twenty one cyathostomin species have been characterized by RLB assay [27]**.** Assays like these exclude the use of classical morphological identification, which are time-consuming, need special skills, and applied on the adult stage only. As both the RLB assay and the PCR-ELISA can be applied on any parasitic stage [26]**.** Traversa et al. [77] applied the RLB assay to identify thirteen species of cyathostomins (equine small strongyles) and discriminated them from three large strongyles *(Strongylus* spp: *S. edentatus*, *S. equinus*, and *S.vulgaris*) by. This RLB method explain some aspects of cyathostominosis and promises to be an excellent diagnostic technique of individual species in the pathogenesis of mixed infections [77].

Recently, two diagnostic methods, RT- PCR and larval culture –method, for the detection of infections with *S. vulgaris* in equine faecal samples were compared [78]. The RT-PCR demonstrated that DNA of *S. vulgaris* was 1.9 % in ten of 501 equine samples. However, the larval culture revealed 1.1% larvae of *S. vulgaris* in three of the 278 samples. The RT-PCR should consequently be considered as a good diagnostic tool for *S. vulgaris* in equine samples .Also, Kandil et al. [79] investigated the genetic diversity among and within *H. contortus* in Egypt by PCR technique. *H. contortus* causes significant economic losses in small ruminants worldwide. PCR technique revealed that all worms have one genotype (*ITS2*) without genetic differentiation. This result could have implications for the rapid characterization of *H. contortus* and other trichostrongyloid .

Diagnosis of Cestodes based on molecular techniques

PCR technique have been applied for diagnosis of *T.saginata* and *T.solium* from different geographical locations [80]. This PCR analysis of DNA isolates confirmed morphologic diagnosis with proportionate and clear inter-species differences between *T. solium* (3 samples) and *T. saginata* (22 samples) isolates. Within these species, possible intra-species genomic variability was similarly studied through PCR-RFLP and only one *T. saginata* isolate from Kenya was performed, different from *T. saginata* DNA of Spanish (7 samples) origin and Mexican (1 sample). Also, a nested PCR have been applied for *T. solium* DNA detection. The assay's specificity and sensitivity were 100% and 97%, respectively with archived samples. However, both the sensitivity and the specificity of the assay were 100% when the nested PCR was tested in the field [80].

Also, molecular techniques can consider a valuable tool in the study of *E. granulosus* epidemiology. Boubaker et al. [81] used PCR assay to detect the genus *Echinococcus*. The genetic variation of *Echinococcus* species can reflect contrasts in infectivity for specific host species. Hence it is of huge significance to phylogenetically portray *E. granulosus* population structure [82]. Omar et al. [83] presented the molecular characterization of *C. tenuicollis* of *T.hydatigena* from livestock isolates in Egypt. PCR assay revealed that there was high similarity between sheep and goat samples (the 340 base pair fragment that corresponds to the mitochondrial *CO1* gene).While more frequently differences were found in the camel samples (10 bp). Obviously, diagnosis for *C. tenuicollis* infection by molecular technique helps to differentiate it from such other metacestodes as hydatidosis, which requires different control programs.

Determination of the genotypes of *E. granulosus* in farm animals of Egypt and Italy have been done by Kandil et al. [84]. The rapid diagnosis and characterization of *E. granulosus* genotypes were done by a specific and sensitive PCR, semi nested PCR system. Characterization of genotypes G1 for sheep, cattle and goats whereas G6 for camel. This study identified as the *E. granulosus* G1 genotype (from Egypt and Italy), and 2 isolates (both derived from camel in Egypt) belonged to the G6 genotype. These data indicated some epidemiological features and molecular characteristics of *E. granulosus* in Egyptian and Italian farm animals.

Diagnosis of trematode based on molecular techniques

RAPD-PCR assay used to characterize *F. gigantica* isolates from cattle in different localities [85]. This study represented the variability of *F. gigantica* isolates from the same host and using RAPD markers could be applied as a low cost way of identification. Three different methods were applied to diagnose *F. hepatica* infection in naturally and experimentally infected sheep [86]: coprological method, S- ELISA kit assay and standard and nested PCR assays. The percentage of infection at 4 weeks post infection (wpi) was 57.1% then reached 100 % at 8wpi by S- ELISA kit assay. All naturally infected animals were positive with this method. However, the *F. hepatica* infection was 82 % at three wpi with a PCR, and from two weeks with a nested-PCR. This study concluded that the sensitivity of the nested-PCR is higher than the commercial immunoassay. Also, no cross reactions were related with GI nematodes. In addition, Ayaz et al. [87] demonstrated that prevalence of fasciolosis in buffaloes and cattle was higher in abattoir of district in Pakistan and PCR was a more sensitive method of diagnosis than microscopy. Identification and differentiation of the two species of *Fasciola* for epidemiological applications. Molecular assay to differentiate between both *F. gigantica* and *F. hepatica* in cattle and sheep has been applied [88, 89]**.** PCR and sequencing amplicons revealed that there was no variation of the 18s rRNA sequence among the multiple samples from cattle and sheep if compared to the corresponding sequences in the gene bank. However, six nucleotide differences were detected between *F. gigantica* and *F. hepatica* isolated in Egypt. These differences among the *Fasciola* spp. can be utilized as molecular markers for diagnosis of fascioliasis in Egypt [89] **.**Also, a molecular methods based on the detection of *F. hepatica* DNA in faeces which collected from natural infected cattle and sheep were applied by Arifin et al. [90]. Arifin et al. [90] applied LAMP and the performance of PCR in diagnosis of *F. hepatica* from naturally infected sheep and cattle (53 animals). In this study, the serology coproantigen ELISA (cELISA), and the outcomes of faecal egg count (FEC), were compared with LAMP and the performance of PCR in diagnosis of *F. hepatica*. DNA- faeces samples were examined both by LAMP and PCR. This results revealed that only 6 and 3 samples were positive by LAMP and PCR, respectively.

Infection of GIT with *Paramphistomum spp* has a great prevalent in domestic ruminants worldwide. Paramphistomosis infections resulting in morbidity, mortality, and reduced meat, wool, and milk production. Approximately 40 species of paramphistomes have been reported**,** but the dominant species are *Gastrothylax crumenifer, Gigantocotyle explanatum, Paramphistomum cervi*, and *Fischoederius elongates* [91, 92]. The identification based on morphological features of these trematode helminths is very difficult [93]. To discriminate among different species a molecular characterization is necessary. Polymorphic DNA fingerprint analysis of three different species of paramphistomes isolated from the rumen and bile ducts of buffaloes were performed. The ruminal paramphistomes were identified as *P.cervi* and *G. indicus*, while the hepatic paramphistomes were identified as *G.bathycotyle*. The RAPD fingerprint suggested close relatedness between

G. bathycotyle and *G. indicus* as compared to *P. cervi*. This study concluded that the RAPD can be used successfully to identify various species of parasite and it is a simple way of creating genomic DNA 'fingerprints' [93].

Conclusion

Helminths infection of the GIT involve the three main groups: nematodes, cestodes and trematodes. Although the traditional methods of diagnosis are specific, they are time consuming, laborious and lacks sensitivity especially in case of light infection. ELISA and its modifications were the commonly used assay in detecting host immune responses and parasite antigens together with western blotting. In addition, advanced molecular techniques are excellent and recommended for laboratory-based research that lead to improve the accuracy and sensitivity of helminths identification and characterization. The accurate diagnostic techniques, immunological and molecular, are urgently needed not only for diagnosis but also for treatment follow up.

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References

1. Chaturvedi, M., Dwivedi, S. and Dwivedi, A. Formulation and evaluation of polyherbal anthelmintic preparation. *Pharmacol . Online,* 13,329-331 (2009).
2. Deore, S.L, Khadabadi, S.S., Kamdi, K.S. and Ingle, V.P., In vitro anthelmintic activity of *Cassia tora*. *Int. J. Chem. Tech. Res*., 1(2), 177-179 (2009).
3. Perry, B. D. Investing in animal health research to alleviate poverty. ILRI (International Livestock Research Institute), Nairobi, Kenya, pp.148 (2002).
4. Schmidt, G.D. and Roberts, L.S. Superorder: Epitheliocystidia: orders Plagiorchiata and Opisthorchiata pp. 308-330 *in* Schmidt, G.D. and Roberts, L.S. (Eds) Foundations of parasitology. 2nd edn Missouri,C.V. Mosby Co., (1981).
5. Sykes, A.R. Parasitism and production in farm animals. *Anim. Prod.,* **59**,155-172 (1994).
6. Kebede, B., Sori, T. and Kumssa, P. Review on current status of vaccines against parasitic diseases of animals. *J. Vet. Sci. Techno.,* **7**(3), 1000327 , pages 1-9(2016). DOI:10.4172/2157-7579.1000327
7. Abouzeid, Z., Selim, M. and El-Hady, M. Prevalence of GI parasites infections in sheep in the Zoo garden and Sinai district and study of the efficacy of anthelmintic drugs in the treatment of these parasites. *J. Am. Sci.,* 6, 544–551(2010).
8. Sultan, K., Elmonir, W. and Hegazy Y. GI parasites of sheep in Kafrelsheikh governorate, Egypt: Prevalence, control and public health implications .*JBAS,* **5**, 79–84 (2016).
9. Lobos-Ovalle , D., Navarrete, C . , Navedo, J. G. and Peña-Espinoza, M. Improving the sensitivity of gastrointestinal helminths detection using the Mini-FLOTAC technique in wild birds. *Res. square*, **1**,1-10 (2021). DOI: https://doi.org/10.21203/rs.3.rs-370319/v1
10. Ndao, M., Diagnosis of parasitic diseases: old and new approaches Interdiscip. Perspect. *Infect. Dis.,* **2009** (Article ID 278246), 15 (2009).
11. Abdul Razzaq, A. K., Maqbool, A., I. M., Abdul Hanan, A. M.M., Khetran ,M.A. , Jan, S., Shafee, M. , Essa, M. and Kakar, H. Epidemiology, sero-diagnosis and therapeutic studies on nematodes infection in Balochi range-sheep at District Quetta, Balochistan, Pakistan. *Iran. J. Parasitol.,* **9**,169-180 (2014).
12. Amarante, A.F.T. and Amarante, M.R.V. Advances in the diagnosis of the GI nematode infections in ruminants. *Braz. J. Vet. Res. Anim. Sci.,* **53**,127-137(2016).
13. Ellis, T.M., Gregory, A., Turnor, R., Kalkhoven, M. and Wroth, R.H. Detection of *H. contortus* surface antigen in faeces from infected sheep. *Vet. Parasitol.,* **51**, 85–97 (1993).
14. Johnson, P.C., Webster, L.M., Adam, A., Buckland, R., Dawson, D.A. and Keller, L.F. Abundant variation in microsatellites of the parasitic nematode *Trichostrongylus tenuis*
15. Agneessens, J., Claerebout, E. and Vercruysse, J. Development of a copro-antigen capture ELISA for detecting *Ostertagia ostertagi* infections in cattle. *Vet Parasitol.,* **97**, 227–238 (2001).
16. Ananda, K. and Gowda, J. Latex agglutination test - a rapid diagnostic technique for the detection of *H. contortus* infection in sheep. *Indian Vet. J.,* **93**, 19 – 21 (2016).

17. Sultan, K., Desouky, A.Y., EL Bahy, N.M. and EL Siefy, M.A. Evaluation of indirect ELISA in diagnosis of natural ovine cysticercosis and haemonchosis. *J. Anim. Feed Res.*, 2,301-302 (2012).

18. Pawlowski, Z.S. and Murrell, K.D. Taeniasis and cysticercosis. In: Food borne disease handbook, ed. Hui YH, Sattar SA, MurrellKD, 2nd ed., pp. 217–227. Marcel Dekker, New York (2001)..

19. Ogunremi, O. and Benjamin, J. Development and field evaluation of a new serological test for *T. saginata* cysticercosis. *Vet. Parasitol.,* **169**(1-2), 93-101 (2004).

20. El Ridi, R., Salah, M., Wagih, A., William, H., Tallima, H., El Shafie, M.H., Abdel Khalek, T., El Amir, A., Abo Ammou, F.F. and Motawi H. *F. gigantica* excretory-secretory products for immunodiagnosis and prevention of sheep fasciolosis. *Vet. Parasitol.,* **149**, 219-228. (2007).

21. Renneker, S., Kullmann, B., Gerber S., Dobschanski, J., Bakheit, M.A., Geysen D. Development of a competitive ELISA for detection of *Theileria annulata* infection. *Trans bound Emerg. Dis.*, **55**, 249–256 (2008).

22. Nkouawa, A., Sako, Y., Li T., Chen X., Wandra, T., Swastika, I.K. and Nakao, M. Evaluation of a loop-mediated isothermal amplification method using fecal specimens for differential detection of *Taenia* species from humans. *J. Clin. Microbiol.,* **48** (9), 3350-2 (2010).

23. Santos, L. L. , Salgado, J A. , Drummond, M. G. , Bastianetto, E., Santos, C. P., Brasil, B. S. A. F. , Taconeli C.A. and Oliveira, D. A. A. Molecular method for the semiquantitative identification of gastrointestinal nematodes in domestic ruminants. *Parasitol. Res*., **119**:529–543 (2020).

24. Gasser, R.B. Molecular tools – advances, opportunities and prospects. *Vet. Parasitol.,* **136**(2), 69-89 (2006).

25. Love, S., Murphy, D. and Mellor, D. Pathogenicity of cyathostom infection. *Vet. Parasitol.,* **85**,113–121(1999).

26. Ionita, M., Howe, D.K., Lyons, E.T., Tolliver, S.C., Kaplan, R.M., Mitrea, I.L. and Yeargan, M. Use of a reverse line blot assay to survey small strongyle (Strongylida: Cyathostominae) populations in horses before and after treatment with ivermectin. *Vet. Parasitol.*, **168**, 332–337 (2010).

27. Cwiklinski, K., Kooyman, F.N.J., Van Doorn, D.C.K., Matthews, J.B. and Hodgkinson, J.E. New insights into sequence variation in the IGS region of 21 cyathostomin species and the implication for molecular identification. *J. Parasitol.*, **139** (8),1063-1073(2012). DOI: https://doi.org/10.1017/S0031182012000467

28. Learmount, J. , Conyers, C., Hez Hird, Morgan, C., Craig, B.H., Samson-Himmelstjerna, G. and Taylor, M.. Development and validation of real-time PCR methods for diagnosis of *Teladorsagia circumcincta* and *H. contortus* in sheep. *Vet. Parasitol*., **166** (3–4), 268–274 (2009).

29. Paris, D.H., Imwong, M., Faiz, A.M., Hasan, M., Yunus, E.B. and Silamut, K. Loop-mediated isothermal PCR (LAMP) for the diagnosis of *falciparum malaria*. *Am J Trop Med Hyg.,* **77**(5), 972-6 (2000).

30. Mori, Y., Nagamine, K., Tomita, N. and Notomi T. Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem. Biophys. Res. Commun.,* **289** (1), 150-4 (2001).

31. Temperley, N.D., Webster, L.M., Adam, A., Keller, L.F. and Johnson, P.C. Cross-species utility of microsatellite markers in *Trichostrongyloid nematodes*. *J. Parasitol.,* **95**(2), 487-489 (2009).

32. Li, X., Du, A., Cai, W., Hou, Y., Pang L. and Gao, X.. Evaluation of a recombinant excretory-secretory *H. contortus* protein for use in a diagnostic ELISA. *Exp. Parasitol.*, **115**, 242–246 (2007).

33. Demeler, J., Schein E. and Von Samson-Himmelstjerna, G. Advances in laboratory diagnosis of parasitic infections of sheep. *Vet. Parasitol.,* **189**2012) .64–52 ,) 1 ().

34. Lind, E.O., Rautalinko, E., Uggla, A., Waller, P.J., Morrison, D.A. and glund, J. Parasite control practices on Swedish horse farms. *Acta. Vet. Scand.,* **49**(1):**25**(2007). doi: 10.1186/1751-0147-**49-25**.

35. Fritzen, B., Rohn, K., Schnieder, T. and von Samson-Himmelstjerna, G., Endoparasite control management on horse farms – lessons from worm prevalence and questionnaire data. *Equine Vet. J*., **42**, 79–83 (2010).

36. McManus, D.P. Immunodiagnosis of sheep infections with *Echinococcus granulosus*. *Parasite Immunol,*. **36**,125–130 (2014).

37. Dowdall, S.M.J., Matthews, J.B., Mair, T., Murphy, D., Love, S. and Proudman C.J. Antigen-specific IgG (T) responses in natural and experimental cyathostomin infection in horses. *Vet. Parasitol.,* **106**, 225–242 (2002).

38. Arab, R. M.H., Abu El Ezz, N. M.T. , Deghidy, N. S. , Awed, W. S.A. and Hasssan N.M.F. Protective value of *H. contortus* adult worm purified antigen against haemonchosis in sheep. *GV*., 11, 614-621 (2013).

39. Kandil, O.M., Hendawy, S.H., El Namaky, A.H., Gabrashanska, M.P. and Nanev, V.N. Evaluation of different *Haemonchus contortus* antigens for diagnosis of sheep haemonchosis by ELISA and their cross reactivity with other helminths. *J. Parasit. Dis.,* **41**, ٦٧٨–683(2017).

40. Abo aziza, F., Hendawy S. M., El Namaky, A. H. and Ashry, H. Th1 / Th2 balance and humoral immune response to potential antigens as early diagnostic method of equine *Strongylus* nematode infection. *Vet. World J.,* 1-10 (2017).

41. Yamane, K., Suzuki, Y., Tachi, E., Li, T., Chen, X. and Nakao, M. Recent hybridization between *T. asiatica* and *T. saginata*. *Parasitol. Int.,* **61**(2), 351-355 (2012).

42. Lightowlers, M.W. and Gottstein, B. Echinococcosis/ hydatidosis: antigens, immunological and molecular diagnosis. In Thomson RCA & Lymbery AJ (eds): The biology of *Echinococcus* and hydatid disease. Walling ford, Oxon, UK, CAB International, 355–419 (1995).

43. Abdel-Rahman, E.H., Abdel-Megeed, K.N., Abuel-Ezz, N.M.T. Cross-reaction: A common trait among Helminths. *J. Egypt. Soc. Parasitol.,* **33**, 457-471 (2003).

44. Yong, W.K., Heath, D.D. and Van Knapen, F. Comparison of cestode antigens in an enzyme-linked immunosorbent assay for the diagnosis of *E. granulosus*, *T. hydatigena* and T ovis infections in sheep. *Res. Vet. Sci.,* **36**, 24–31(1984).

45. Jenkins, D. J. and Rickard, M. D. Specificity of scolex and oncosphere antigens for the serological diagnosis of taeniid cestode infections in dogs. *Aust. Vet. J*. **63**:40–42 (1986).

46. Burgu, A., Douanay, A.B.G., Sarimehmetoulu, H.O. and Kalinbacak, F. Analysis of fluids of hydatid cysts from sheep by SDS-PAGE, and determination of specific antigens in protein structure by western blotting. *Turk. J. Vet. Anim. Sci*., **24**,493-500 (2000).

47. Kittelberger, R., Reichel, M.P., Jenner, J., Heath, D.D., Lightowlers, M.W., Moro, P., Ibrahem, M.M., Craig, P.S. and O'Keefe, J.S.,. Evaluation of three enzyme-linked immunosorbent assays for the detection of serum antibodies in sheep infected with *E. granulosus. Vet. Parasitol*., **110,** 57–76 (2002).

48. Jeyathilakan, N., Abdul Basith, S., John, L., Chandran, N.D.J., Raj, G.D. Evaluation of native 8 kDa antigen based three immunoassays for diagnosis of cystic echinococcosis in sheep. *Small Ruminant Res.,* **116**, 199-205 (2014).

49. Sangaran, A., Bino Sundar, S. T. and Latha, B. R. Antigen based detection of cystic echinococcosis in buffaloes using ELISA and Dot-EIA. *J. Para. Dis*., 41(1), 128–130 (2017).

50. El Shazly, A.M , Saad, R.M., Belal, U.S., Sakr, T. and Zakae, H.A. , Evaluation of ELISA and IHAT in serological diagnosis of proven cases of human hydatidosis. *J. Egypt. Soc. Parasitol.,* **40**, 531-538 (2010).

51. Ramadan N.I., Abel Aaty, H.E., Mahmoud, M.S. and El Nori, A. An enzyme-linked immune-electro transfer blot assay for diagnosis of human cystic echinococcosis. *J. Egypt Soc. Parasitol.,* **29**, 849-857 (1999).

52. Mahmoud, M.S., Derbala, A.A., El-Massry, A.A. and Maarouf, O.A. Sero- diagnostic potency of hydatid fluid and protoscoleces partially purified fractions of both camel and equine origin. *GV.,* **2**, 99-103 (2008).

53. Al-Olayan, E.M. and Helmy, H. Diagnostic value of different antigenic fractions of hydatid cyst fluid from camel and sheep in Kingdom of Saudi Arabia. *J Saudi Chem. Soc*., **16**, 203–207 (2012).

54. Al-Sherbiny, M.M., Farrag, A.A., Fayad, M.H., Makled, M.K.,Tawfeek, G.M. and Ali, N.M. Application and assessment of a dipstick assay in the diagnosis of hydatidosis and trichinosis. *Parasitol. Res.,* **93**, 87-95 (2004).

55. Hassanain, M.A., Shaapan, R.M. and Khalil, F.A.M., Sero- epidemiological value of some hydatid cyst antigen in diagnosis of human cystic echinococcosis. *J. Parasit. Dis.,* **40**, 52-56 (2016).

56. Kandil, O.M., Mahmoud, M. S. Shalaby, H.A., El Namaky, A. H., Hendawy, S.H.M. and Arafa M.I. Value of *T. Saginata* crude antigen in diagnosis of bovine cysticercosis with reference to its characterization. *GV.,* **9** (4), 474-478 (2012).

57. Salimi-Bejestani, M.R., McGarry, J.W., Felstead, S., Ortiz, P., Akca, A. and Williams, D.J.L. Development of an antibody-detection ELISA for *F. hepatica* and its evaluation against a commercially available test. *Res. Vet. Sci.,* **78**, 177–181(2005).

58. Mandal, S., Mukhopadhayay, S.K., Ganguly, S. and Jana, S.,. Immunodiagnosis as an aid for early detection of *Fasciola gigantica* by glutathione *S*-transferase (GST). *J. Parasit. Dis*., 36, 207–209 (2012).

59. Anuracpreeda, P., Chawengkirttikul, R. and Sobhon, P., . Immunodiagnostic monoclonal antibody-based sandwich ELISA of fasciolosis by detection of *Fasciola gigantica* circulating fatty acid binding protein. *Parasitol*2016) 1-13 ,**17** ,.).

60. Shalaby, S.I., El-Bahy, M., Shalaby H., Gupta N., Gupta D.K., Detection of coproantigens by sandwich ELISA in rabbits experimentally infected with *F. gigantica*. *Iran J. Parasitol.,* **9**,374-81(2014).

61. Guy, R.A., Xiao, C. and Horgen, P.A. Real-time PCR assay for detection and genotype differentiation of *Giardia lamblia* in stool specimens. *J. Clin. Microbiol.*, **42**(7),3317-20 (2004).

62. Farcas, G.A., Soeller, R., Zhong, K., Zahirieh, A. and Kain, K.C. Real-time polymerase chain reaction assay for the rapid detection and characterization of chloroquine-resistant *Plasmodium falciparum* malaria in returned travelers. *Clin. Infec. Dis.,* **42**(5), 622-7(2006).

63. Monis, P.T. and Andrews, R.H. Molecular epidemiology: assumptions and limitations of commonly applied methods. *Int. J. Parasitol.*, **28**(6), 981-987(1998).

64. Jain, S.K., Neekhra, B., Pandey, D. and Jain, K. RAPD marker system in insect study: a review. *Indian J. Biotechnol*., **9** (1), 7-12 (2010).

65. Martinez, E.M., Correia, J.A., Villela, E.V., Duarte, A.N., Ferreira, L.F. and Bello, A.R. Random amplified polymorphic DNA analysis of DNA extracted from *Trichuris trichiura* (Linnaeus, 1771) eggs and its prospective application to paleo parasitological studies. *Mem. Inst. Oswaldo. Cruz*., **1**, 59-62 (2003).

66. Nuchprayoon, S., Junpee, A. and Poovorawan Y., Random amplified polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of *Wuchereria bancrofti*. *Filaria J*., **6** (1), 2007) 1-6.

67. Sharbatkhori, M., Mirhendi, H., Harandi, M.F., Rezaeian, M., Mohebali, M. and Eshraghian, M.,. *E. granulosus* genotypes in livestock of Iran indicating high frequency of G1 genotype in camels. *Exp Parasitol.,* **124**(4), 373-379 (2010).

68. Bobes, R.J., Fragoso, G., Reyes-Montes, M.R., Duarte-Escalante, E., Vega, R.and de Aluja, A.S. Genetic diversity of *T.solium cysticerci* from naturally infected pigs of central Mexico. *Vet. Parasitol.,* **168** (1-2), 130-135 (2010).

69. Alimoradi, S., Hajjaran, H., Mohebali, M. and Mansouri F. Molecular identification of *Leishmania* species isolated from human cutaneous leishmaniasis by RAPD-PCR. *Iranian J. Publ. Health.,* **38**(2), 44-50 (2009).

70. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K. and Amino N. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.,* **28** (12), E63 (2000).

71. Ai, L., Li, C., Elsheikha, H.M., Hong, S.J., Chen, J.X. and Chen S.H. Rapid identification and differentiation of *F. hepatica* and *F.gigantica* by a loop-mediated isothermal amplification (LAMP) assay. *Vet. Parasitol.,* **174**(3-4), 228-233 (2010).

72. Carpentieri-Pípolo, V., Gallo-Meagher, M., Dickson, D.W., Gorbet, D.W., Mendes, M.L. and de Souza, S.G.H. Comparação entre métodos de marcação da sonda de RFLP R2430E utilizada na seleção de cultivares de amendoim resistente à *Meloidogyne arenaria*. *Ciênc. Agrár.,* **29**(4), 783-788 (2008).

73. Zaeemi, M., Haddadzadeh, H., Khazraiinia, P., Kazemi, B. and Bandehpour, M. Identification of different *Theileria* species (*Theileria lestoquardi*, *Theileria ovis*, and *Theileria annulata*) in naturally infected sheep using nested PCR-RFLP. *Parasitol. Res.,* **108** (4), 837- 843 (2011).

74. Silva, L.M. , Miranda, R.R., Santos, H.A. and Rabelo, E.M.. Differential diagnosis of dog hookworms based on PCR-RFLP from the ITS region of their rDNA. *Vet. Parasitol*., **140** (3-4), 373-377 (2006).

75. Oliveira, E.J., Pádua, J.G., Zucchi, M.I., Vencovsky, R. and Vieira, M.L.C., Origin evolution and genome distribution of microsatellites. *Genet. Mol. Biol.*, **29**(2), 294-307 (2006).

76. Bott, N.J. , Campbell, B.E., Beveridge, I., Chilton, N.B. , Rees, D. and Hunt, P.W. A combined microscopic-molecular method for the diagnosis of strongylid infections in sheep. *Int. J. Parasitol.,* **39**(11), 1277–1287 (2009).

77. Traversa, D., Iorio, R., Klei, T.R., Kharchenko, V.A., Gawor, J., Otranto, D. and Sparagano, O.A. New method for simultaneous species-specific identification of equine strongyles (nematodastrongylida) by reverse line blot hybridization. *J. Clin. Microbiol.,* **45**, 2937–2942 (2007).

78. Kaspar, A., Pfister, K., Nielsen, M.K., Silaghi, C., Fink, H. and Scheuerle M.C. Detection of *S. vulgaris* in equine faecal samples by real-time PCR and larval culture – method comparison and occurrence assessment .*Vet. Res.,* **13**(1), 19 (2017). doi: 10.1186/s12917-016-0918-y.

79. Kandil, O.M., Abdelrahman, K.A., Fahmy, H.A., Mahmoud, M.S., El Namaky, A.H. and Miller J.E. Phylogenetic patterns of Haemonchus contortus and related trichostrongylid nematodes isolated from Egyptian sheep. *J. Helminthol.*, **91**(5):583-588 (2016).

80. Gonza, L.M., Monteroa, E., Puenteb, S., Lo´pez-Velezc, R., Herna´ndezd, M., Sciuttod, E., Harrisone, L. J.S., Parkhousef, R. M. E. and Ga´ratea T. PCR tools for the differential diagnosis of *T. saginata* and *T. solium* taeniasis/cysticercosis from different geographical locations. *Diagnos. Microbiol. Infec. Dis.,* **42**, 243–249 (2002).

81. Boubaker, G., Macchiaroli, N. and Prada L. A Multiplex PCR for the Simultaneous Detection and Genotyping of the *E. granulosus* Complex. *PLoS Negl. Trop. Dis.*, **7**, 20-17 (2013).

82. Thompson, R. C. A and McManus, D P. Towards a taxonomic revision of the genus Echinococcus. *Trends Parasitol.*, **18**,452– 457(2002).

83. Omar, M.A., Elmajdoub, L.O., Al-Aboody, M.S., El Sify, A.M., Elkhtam, A.M. and Hussien, A.A.. Molecular characterization of *Cysticercus tenuicollis* of slaughtered livestock in Upper Egypt governorates .*Asi. Pac. J. Trop. Biomed*., **6** (8), 706–708 (2016).

84. Kandil, O.M., Abdelrahman, K.A, Abu El Ezz, N.M.T. and Antonio, V. Genetic Diversity of *E. granlosus* isolated from farm animals by using nuclear and mitochondrial genetic loci. *Int. J. Chem.Tech. Res.,* **9**, 169-177 (2016).

85. Chauke, E., Dhlamini, Z., Mbanga, J. and Dube, S. Characterization of *F. gigantica* isolates from cattle from south-western Zimbabwe using RAPD-PCR. *J. Agri. Vet. Sci*., **7** (2), 19-25 (2014).

86. Martínez-Pérez, J.M., Robles-Pérez, D., Rojo-Vázquez, F.A.› Martínez-Valladares M. Comparison of three different techniques to diagnose *F. hepatica* infection in experimentally and naturally infected sheep. *Vet. Parasitol.*, **190** (1–2), 80–85 (2012).

87. Ayaz, S., Riaz, U., Naser, M. A., Sumiara, S. and Sadaf, N. *F. hepatica* in some buffaloes and cattle by PCR and microscopy. *Sci. World J.*, **2014**, 1- 5. (2014).

88. El-Rahimy, H.H, Mahgoub, A.M.A., El-Gebaly, N.S.M., Mousa, W.M.A. and Antably, A.S. Molecular, biochemical, and morphometric characterization of Fasciola species potentially causing zoonotic disease in Egypt. *Parasitol. Res.,* **111**, 1103–1111 (2012).

89. Ayoub, M.B., Wahba, A.A. and Ibrahim, M. Molecular characterization of *Fasciola* spp. in sheep and cattle. *Ani. Heal. Res. J.,* **3** (267-75 ,) 2015)).

90. Arifin, M.I., Höglund, J. and Novobilský, A. Comparison of molecular and conventional methods for the diagnosis of *F.hepatica* infection in the field. *Vet. Parasitol*., **232**, 8-11(2016).

91. Agrawal, M.C. . Helminthology in India. Dehradun, ISBN 10: 8170892937 , India: International Book Distributor, (2003).

92. Hassan, S.S., Kaur, K., Joshi, K. and Juyal, P.D Epidemiology of paramphistomosis in domestic ruminants in different district of Punjab and other adjoining areas. *J. Vet. Parasitol*., **19**, 43–46 (2005).

93. Puttalakshmamma, G., Ramaniu, U., Singh, K., Patel, A. and Joshi, C. Genetic characterization of paramphistomes of buffalo by HAT-RAPD analysis. *Turk. J. Vet. Ani. Sci*., **38**, 7-13 (2014).

التقنيات المتقدمة في تشخيص الديدان الطفيلية للماشية

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تعد الإصابة بالديدان الطفيلية من أكثر الأمراض شيوعاً وتشكل عائقاً رئيسياً للإنسان والحيوان وتؤدي إلى سوء التغذية وفقر الدم وأيضاً الالتهاب الرئوي. تشتمل عدوى الجهاز الهضمي بالديدان الطفيلية ثلاث مجموعات: الخيطيات (النيماتودا)، الشريطيات (السيستود) والمفلطحات (التريماتودا). تسبب الإصابة بالديدان الخيطية والشريطية إلى التهاب حاد في المعدة والأمعاء كما تسبب الإصابة بالتريماتود إلى ضرر بالغ للكبد وانسداد للقنوات المرارية. تحدث الإصابة بمعظم الديدان المعوية عن طريق الفم ولكنها تختلف في العائل الوسيط والنهائي. يتم تشخيص الإصابة باستخدام الوسائل التقليدية إما عن طريق المسحة المباشرة للعينة أو عن طريق استخدام تقنية الترسيب/التعويم وتستغرق طرق الفحص التقليدية وقتاً طويلاً كما أن حساسيتها منخفضة في الكشف عن درجة الإصابة.

يعتبر التشخيص باستخدام الوسائل المناعية مثل تقنية الاليزا وتعديلاتها من التقنيات المناسبة.حيث أظهرت تقنية الاليزا فعالية في تشخيص الإصابة بديدان الهمونكس كونترتس وديدان التليدورساجيا في الأغنام وديدان أوستيرتجيا في الأبقار. كما أظهرت تقنيات الاليزا غير المباشرة وأيضاً الطبع المناعي (WB) حساسية بالغة في الكشف عن الإصابة بديدان الاسترونجليس والإكينوكوكس والفاشيولا في كلاً من الأغنام والخيول والأبقار والجاموس.

أيضاً يعتبر التقدم في وسائل التشخيص باستخدام البيولوجيا الجزيئية مثل تفاعل البلمرة المتسلسل (PCR)، وتقنية النسخ العكسي (RLP)،تقنيه التضخيم الحراري المتساوي الحلقة (LAMP)، وتقنية تفاعل البلمرة المتسلسل محدد الوقت (RT-PCR)، وهذه التقنيات أظهرت فعالية وحساسية بالغة في الكشف والتشخيص الدقيق وتميز الحمض النووي (DNA) للعديد من الطفيليات. ويعتبر استخدام تقنية تفاعل البلمرة المتسلسل أو مجتمعة مع التقنيات الأخرى مثل RT-PCR، وتقنية LAMP أو RLP أظهرت فعالية عالية للكشف عن التركيب الوراثي لكل طفيل في البراز وتعتبر هذه التقنيات أكثر دقة وذلك بالمقارنة باستخدام المجهر الضوئي. حيث أوضحت تقنية LAMP دقة في تشخيص الإصابة بديدان التينيا في عينات البراز وأيضاً تميز أنواع ديدان الفاشيولا من خلال الحمض النووي الخاص بكل نوع. ويعتبر التشخيص باستخدام تقنية RLB من التقنيات بالغة الدقة حيث أنه يمكن تحديد الأنواع المختلفة من الإصابة بالديدان في العدوى المختلطة كما في حالة الإصابة بديدان الثيائوستومينس في الخيول.

وأخيراً فهناك حاجة ملحة إلى استخدام التقنيات الحديثة ليس فقط للتشخيص ولكن أيضاً لمتابعة العلاج.