"IMMUNO-INTER-RELATION BETWEEN SOME HELMINTHES OF SHEEP"

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I. Introduction

In Egypt, sheep have been considered as one of the most promising animals to achieve the aims of animal products supplies for the human being, also small ruminants meat considered as a major part of the red meat consumption and represent 17% of total red meat consumption (**Haenlein and Abdellatif, 2003**) in addition to sheep manure consider as a natural organic fertilizer for the agriculture lands.

Infection of sheep with different species of helminthes considered as a great hazard that seriously interferes with sheep productivity improvement, More than 20 different species of helminthes belonging different categories may infect sheep which may act as final host or intermediate host of these helminthes.

Fasciola gigantica is one of the most important parasites in sheep, the disease charachterised by loss of weght, anemia and hypoprotenemia which subsequently leads to loss of productivity either meat, milk or wool and animal fertility, nevertheless in heavy infestations of sheep sudden death may occur. (**Solusby, 1982**).

Economic loss due to *Fasciola spp.* infection was estimated to be about US$200 million/year , to rural agricultural communities and commercial producers, (**Spithill and Dalton, 1998**), whereas in Egypt, Losses were estimated to be about L.E. 100 million per year this fact recorded by (**Egyptian Academy of Scineftic Research and Technology, 1990**).

Moneizia species inhabit the small intestines of sheep causing a varitey of symptoms including dairrhoea, emaciation and death may occurs either due to cumulative effect of the tapeworms or due to acute toxemia (**Solusby, 1982**), whereas, sheep act as intemediate host for several species of cestode parasites, and so ovine cysticercosis is considered as one of the most wide spreading parasitic diseases infecting sheep allover the world, especially in the developing countries that have a

problem of stray dogs which consider that final host of the adult *Taenia spp.*, a special importance that it has no ante-mortem clinical signs, but detected only during postmortem examination, so its diagnosis by ordinary methods such as coprological examination is helpless **(El-Massry, 1988)**.

Gastrointestinal nematode infections of ruminants cause considerable economic loss throughout the worl **(Miller, 1984)**, especially infection with *Haemonchus contortus.*

Such as infection may leads to anorexia and reduced feed intake, loss of blood and plasma proteins into the gastrointestinal tract, alterations in protein metabolism, depressed levels of minerals, depressed activity of some intestinal enzymes and diarrhea; all contribute to depressed weight gain, wool groth and milk production **(Solusby, 1982)**.

Production of vaccines or diagnostic kits for parasites based on immunological reaction is still the hope of many researchers and livestock herds producer allover the world, since the copmlex strucure of parasites interferes with commercial production of such products, Immuno-inter-relations between different species of enterichelmintheswere approved by many authors **Campbell et al,(1977), Dieen *et al*, (1978), Rajasekariah *et al,(*1979), El-Massry, (1988) and Warren, (1993)**.

So, this work aimed to:

1. The incidence of enteric helminthes of sheep and their pathological effects on their hosts were taken in consideration.
2. Make an attemp to figure the immune realtionships between some helminthes of sheep i.e. *Fasciola spp., Moneizia spp.*, *C.teniucollis* and *H.contortus.*
3. Determine the sensitivity of some antigenic preparations in diagnosis of such infections.

II- Review of Literatures

2.1. Incidence of different helminthes in sheep:

Hassona, (1979) examined 350 sheep abomasa which collected from different districts in Giza governorate during the different seasons, the results showed that the general infection percentage with abomasal worms among examined sheep was 35.428%, concerning the species which recovered were *Trichostrongylus axei, Haemonchus contortus, Paraponema skrjabini ,Ostertagia trifurcata* and *Ostertagia ostertagi* and the percentages were 42.4, 38.7and 29.0% for the first three species respectively while the last two species were remarkably scanty.

Pathak and Gaur, (1982) examined 810 sheep, 1015 goats and 1040 pigs were examined for the presence of *Cysticercus tenuicollis* to determine the incidence of these parasites, results showed that the the incidence of *C.teniucollis* was found in 37.03% of sheep, 27.29% of goats and 8.30% of pigs, so the rate of infection was higher in sheep than in goats or pigs, moreover a high infection was found in the rainy season, regarding the intensity of infection it was higher in old than in young animals.

Hassan, (1988) parasitologically examined 2100 sheep of different ages in Beni-Suef governorate, results indicated that 55.13% of the examined sheep were infcted by different nematode eggs and the most common type of eggs was of members of family Trichostrongylidae.

Hasslinger and Weber-Werringhen, (1988) noticed that 785 (16.7%) out of 4.710 slaughtered sheep harboured metacestodes of T.hydatigena in the omentum maujs of infected ones.

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El-Fauomy, (1989) results of parasitological examination of 600 faecal samples collected from sheep of different ages and in different seasons revealed that the infestation rate with nematodes was high in young animals (3 months up to 1 year) 71.74% of examined sheep, sheep over 2 years 63.15% and in sheep (1-2 year) 61.11%, concerning the total inidence of each species it was 4.5, 10.0, 2.83, 63.5% for *Trichuris*, *Strongyloides*, *Nematodirus* and other strongyles respectively.

Kedees, (1990) stated that out of 34 sheep intestinal tract 22 (64.7%) were infected with helminth parasites, 10 (29.4%) had nematodes, 8 (23.5%) had cestodes and 4 (11.8%) had both types.

El-Sayed, (1991) examined 1450 fecal samples of sheep parasitologically , results revealed *Fasciola* eggs in 311 samples (21.4%), while the abattoir survey showed that 386 (26.6%) animals were harboring *Fasciola gigantica* ,bursate nematodes prevailed in 70.5% of *Fasciola* infected cases *Dictyocaulus filaria* and *Moneizia spp.* were found with an infection rate of 25.4% and 14% respectively, mixed infection with the aforementioned helminthes was the main feature of ovine fasciloiasis, moreover it was noticed that *Fasciola* free sheep were consistently harbored *Cysticercus tenuicollis* on their viscera during abattoir examination.

El-Fauomy, (1992) examine 256 abomasa of sheep slaughtered at Cairo abattoir revealed that 45.7% were harbouring abomasal nematodes, 28.9, 22.20 and 17.18 percent of examined abomasa were infected with *Trichostrongylus spp.*, *Haemonchus contortus* and *Ostertagia spp.* respectively.

Abd El-Miguid, (1998) examined 200 intestinal tracts of sheep, results showed that 144 (72%) of it had different helminthes, 80 (55.56%) had only one species and 64 (44.44%) were infected with more than one helminth parasites, nematode worms were recovered from 112 intestinal tract from 200 examined sheep giving prevalence rate of 56%, of them 92 (82.14%) had only one nematode species and 20 (17.86%) were infected with more than one nematode species, while cestode

worms were recovered from 79 intestinal tract of 200 examined sheep giving a prevalence rate of 39.5%, of them 72(91.14%) had only one cestode species and 9 (8.86%) were infected with more than one cestode species.

Haridy *et al*, (1999) During the period 1994 to 1997, investigated about the overall slaughtered animals in Egyptian abattoirs found that 2.003.200 sheep and goats, 2.624.239 cattle and 3.536.744 buffaloes, whereas the overall rates of fascioliasis were 2.02% for sheep and goats, 3.54% for cattle and 1.58% for buffaloes, taken in consideration.

Khalafallah, (2000) examined 173 sheep after slaughtering, results indicated that 45.1% had an infection with one or more of helminthes, 11 % of it had a mixed infection, regarding the recovered species and its incidence rate were *F.gigantica* (2.9%), *Paramphistoums spp.* (3.5%), *Moneizia expensa* (19.7%), *Avitellina centripunctata* (14.5%), *C.teniucollis* (11.6%), *H.contortus* (3.5%), *Parobonema skrjabini* (2.9%) and *Graphidopis spp.* (2.9%).

Awadalla, (2001) after coprological examination and P.M. examination of 287 fecal sample and 137 slaughtered sheep, stated that the true prevelance of fascioliosis among sheep in Giza Governorate was 11.08%, whereas the prevelance rate by using P.M. examination only revealed that 24 (17.52%) out of 137 sheep harbouring *F.gigantica* infection.

Ibrahim et al, (2002) through examination of 300 liver of slaughtered sheep found that the prevelance rates of *F.gigantica* and larval stages of *T.hyadtigena* were 8.4% and 4.6% respectively.

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Abdel-Maogood,(2005) examined 200 sheep of different breeds after slaughtering for the presence of different metacestodes and observe that 45 (16.98%) animals were harboring *C.teniucollis* attached to omentum, peritoneum and liver, while only 15 (5.66%) animals were harbouring *C.ovis* in heart and liver.

Kandil *et al,* (2005) proved that the incidence of infection of sheep with *Cysticercus tenuicollis* (33 cases out of 120 case examined postmortem) higher than *Cysticercus ovis* (only 5 cases out of 120 case examined postmortem), while by ELISA only 21 out of 33 that infected with *C.teniucollis* had IgG antibodies against E/S of *T.hydatigena* antigen, this might be due to the mature cyst is not sufficiently antigenic since very little reaction to metacestodes may be seen due to the presence of omental fat, while the period of migration of larvae from liver o peritoneum corresponds to the higher titer of antibodies.

2.2 Recent immunological techniques in helminth parasites:

Voller, *et al,*(1976) showed that enzyme immunoassays can be applied to many parasitic diseases, the same techniques can of course also be used in other microbial infections such as viral, bacterial and fungal infections, so the fact that a basic technique which is cheap and simple can be used for so many purposes should commend for wide application in the developing parts of the world.

Poluektova,(1983) observe the dynamics of IgM and IgG antibodies in sheep that experimentally infected with *Fasciola hepatica* using indirect haemagglutination test (IHA) using protein fraction as antigen and results showed that specific IgM was detected from day 14 after infection, peak was at day 28 and decline at day 120,while specific IgG detected from day 28, rsoe on days 42 to 87 and decline on day 120 , for more conformation IDT was used with whole *F.hepatica* extract as antigen, but results showed it was not sufficient sensitive to detect specific IgM; IgG from day 28 to 105 .

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Huang *et al,* (1984) used indirect haemagglutination test (IHA) as a way of early diagnosis of fascioliasis, after treated sheep erythrocytes had been sensitized with protein antigen extracts from *Fasciola hepatica* it were used in IHA, of 11 experimental infected sheep, 6 become positive at 2 weeks post inoculation; the other 5 sheep were positive at 3 weeks; they were still positive at the end of the experiment (13 weeks).

Gomez-Arroyo *et al,* (1984) compared the somatic antigens; crude saline (CS) and delipidized saline (DS) of *Fasciola hepatica* in the diagnosis of fascioliasis in sheep using 32 sheep from an endemic zone and 50 sheep seems to be negative by fecal examination and brought from area of low incidence in Mexico, they use the two antigens in the skin test (ID), passive haemagglutination test (HAI), agar double diffusion (DD) and immunoassay (IA); they reach to a result that specificity for the CS was 70 to 96% and 74 to 100% for the DS antigen.

Pfister *et al,*(1984) used partially purified antigen fractions from adult *Fasciola hepatica* (three somatic tissues [Fhs] and three excretory products[Fhm]) in a micro-ELISA, and conclud that excretory-secretory products of a low molecular weight were also antigenic and could differentiate between infected animals and control, the results were inconsistent and further purification of the antigens may eventually improve the sensitivity of the method.

Wang *et al,* (1985) concluded that ELISA is considered suitable for the early diagnosis of *F.hepatica* infection.

Klimenko and Belozerov,(1986) used purified *F.hepatica* proteinase as antigen in ELISA, resulting in 34 (83%) of 41 sera of sheep with natural or experimental fascioliasis react positively and the value rose to 93.3% when only animals experimentally infected with *F.hepatica* were evaluated; no distinction was made between *F.hepatica* and *F.gigantica* in naturally infected sheep.

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Trudgett ***et al,*** **(1988)** by using a monoclonal antibody specific for tegumental antigen of *Fasciola hepatica*, was used as a solid-phase immunosorbent for the purification of T1 antigen from homogenized mature *F.hepatica*, then material fractionated by this method was used to detect antibodies to *Fasciola hepatica* in sera from sheep and cattle, in enzyme-linked immunoassay and this technique was successful.

Santiago and Hillyer, (1988) detect antibodies to *F.hepatica* both somatic and excretory-secretory antigens in two different hosts sera a resistant host (cattle) and a susceptible model (sheep) by using ELISA.

Rivera Marrero ***et al,*****(1988)** decided that the metabolic antigens of *F.hepatica* have been shown to be source of potential immunodiagnostic antigens, so, through fractionation of *F.hepatica* E/S antigens by conventional gel filtration and HPLC, analyzed these fractions in PAGE, then evaluate their immunogenicity by ELISA with sera from experimentally infected rabbits, results showed that a fraction enriched in high molecular weight components of ca. 150-160 kDa was found to be very reactive with sera from early fascioliasis, whereas this fraction was successfully adapted to the DOT-ELISA.

Arriaga de-Morilla ***et al,*****(1988)** conclude that both a crude somatic antigen and a metabolic antigen of adult *F.hepatica* are suitable in immunological diagnosis of fascioliasis in sheep.

Arriaga de-Morilla ***et al,*****(1989)** compare between dot-ELISA, passive haemagglutination test (PHA) and thin layer immunoassay (TIA) in diagnosis of natural or experimental *Fasciola hepatica* infection in sheep, results showed that, dot-ELISA assay is much better than PHA or TIA.

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Bautista-Garfias ***ct al,*** **(1989)** used and made a comparison between the diffusion-in-gel enzyme-linked immunosorbent assay (DIG-ELISA) and the indirect haemagglutination (IHA) technique for serodiagnosis of sheep fascioliasis using adult fluke excretory-secretory products as antigen, whereas both sensitivity and specificity of DIG-ELISA as a serodiagnostic test for sheep fascioliasis were 100%, whereas the sensitivity was 68.2% and the specificity was 100% in the IHA.

Pfister, (1990) decided that although various serological techniques including immunopercipitation, indirect haemagglutination and indirect fluorescence antibody test have been successfully applied for the detection of fascioliasis, mainly in experimental infection, the ELISA assay is a recent and the most frequent used assay utilizing somatic or excretory-secretory products of adult flukes, however, most data indicate that serodiagnosis is reliable in groups of naturally-infected cattle and sheep but cannot yet be applied for the detection of individually infected animals.

Cornelissen ***ct al,*****(1992)** compared indirect haemagglutination test (IHA) and ELISA for the diagnosis of *F.hepatica* in experimentally and naturally infected sheep; serum samples were obtained from Texal sheep aged 3-12 months, 32 having been infected with *F.hepatica* or various nematodes and coccidia, and 157 having grazed infested pasture for 17 weeks, from he results a conclusion that in the ELISA using somatic antigen prepared from adult flukes was 98% specific, and that with excretory-secretory antigen from incubated flukes was 95% specific, compared with 85% for the IHA test; moreover ELISA become positive 2 weeks after infection.

Ruiz-Navarrete ***et al,*** **(1993)** studied during experimental infection the immune response of sheep to somatic components and excretory/ secretory products of adult *Fasciola hepatica*,similarly, the results of Western blot analysis showed specific recognition of several components as early as two weeks after infection, however, an increase in the number and intensity of bands with time of infection was observed in the patterns of antigenic recognition.

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Diaw *et al,* (1994) used ELISA test for diagnosis of *F.gigantica* in sheep in Senegal, study was performed on two 3-month-old Peul-Peul lambs which were orally infected with 300 metacercariae of *F.gigantica*, also the fecal examination were carried out before infection and then weekly from 3rd weeks p.i., results showed that ELISA test is more earlier in diagnosis and more specific than coprological examination.

Ferre *et al,* (1995) detect the seroprevelance of *F.hepatica* infection in sheep in northwestern Spain using standard coprological sedimentation and (ELISA) in which used excretory-secretory products from *F.hepatica* as antigen, the results indicate that the sensitivity of ELISA was 95%, with specificity >99%, although a statically significant association was demonstrated between the mean flock prevalence as determined by ELISA (77.6%) and by coprological examination (23.7%).

Azab and el Zayat,(1996) found that IHA with purified specific antigens both for schistomiasis and fascioliasis gives good specificities and helps to avoid cross-reactions between the two serious emerging trematode infections offering reliable diagnosis.

Martinez *et al,* (1996) found that the ELISA by using excretory –secretory antigens as a technique for the early detection of specific antibodies in *F.hepatica* infections in experimentally infected goats, in all infected goats *F.hepatica* eggs appeared in faeces between 60 and 90 days PI, while antibodies were detected between 15 and 30 days PI and maximum antibody levels were reached at 90 days PI, positive antibody levels wish are significantly differs from those of control were still found at 365 days PI.

Hillyer *et al,* (1996) used ELISA as herd evaluation for determination of *Fasciola hepatica* infection in sheep and cattle, from 184 sheep examined, 22 were positive for *F.hepatica* eggs, while 163 were positive by serology, moreover all of the 22 sheep which were positive parasitologically were also positive serologically for a sensitivity of 100%.

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Mandel ***et al,*** **(1998)** prepare different antigens from *F.gigantica*, somatic antigen (SAg), excretory-secretory antigens (ESAg) and egg antigen (EAg), then these antigens were evaluated for immunodiagnosis of fascioliasis in sheep by using different tests, double immunodiffusion (DID), counterimmunoelectrophoresis (CIEP) and ELISA, in the ELISA ESAg and SAg were the most reactive antigens with no significant difference and EAg was the least antigenic, in CIEP, the EAg showed the highest sensitivity.

Moustafa ***et al,*** **(1998)** performed ELISA for the detection of *Fasciola hepatica* antigens in stool sample (copro-antigens) of experimentally infected animals (mice, rats and rabbits), ELISA test had approved to be a rapid, easy and sensitive test for diagnosis fascioliasis by detecting of *F.hepatica* copro-antigens earlier than routine stool examination of *Fasciola* egg.

Arafa ***et al,*** **(1999)** evaluate *Fasciola* E/S antigens for early diagnosis of human fascioliasis utilizing different diagnostic techniques includes EITB and CIEP, after *Fasciola* specific E/S protein fraction band (49.5 kDa) was determined and electroeluted, the mono-specific antibodies against this specific protein was prepared, the assessment of the prepared mono-specific antibodies performed throughout detection of E/S copro-antigens by ELISA from different patient groups, then sera were collected and tested using EITB and CIEP to detect antibodies against *Fasciola* E/S antigens, it revealed that *Fasciola* E/S 49.5 kDa protein fraction be specific to *F.gigantica*, the conclusion that ELISA assay using mono-specific anti-49.5 kDa to detect copro-antigens proved to be practical and reliable showed high sensitivity (91.4%) and high diagnostic accuracy (91.8%) while sensitivity was 92.3%.

Maleewong ***et al,*** **(1999)** used a continuos-elution method as a simple procedure for obtaining an immunodominant antigens of approximate mass 27 kDa from the excretory-secretory products of adult *Fasciola gigantica*, then by application of an indirect ELISA assay using this antigen for detection of specific antibodies from patients infected with *F.gigantica*, results revealed that he sensitivity,

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specificity, and positive and negative predictive values for this ELISA using the fractionated antigens were 100%.

Carnevale ***et al,*** **(2000)** reach a result that the micro-ELISA could be used as a screening assay and ELISA could be used as a confirmatory method for the serodiagnosis of human fascioliasis, they used excretory-secretory antigens of Fasciola hepatica and the sensitivity of each method was 100%, but the specificity was 100% for ELISA and 97% for micro-ELISA.

El-Aziz ***et al,*** **(2001)** examined 200 blood samples and the corresponding faecal and/or bile of buffaloes, 68 and cattles for *Fasciola* infection, ELISA results showed that ELISA assay using *F.hepatica* excretory-secretory (E/S) antigens is of a diagnostic value for cattle fascioliosis rather than for buffaloes.

Almazan ***et al,*** **(2001)** observe that ELISA detect *F.hepatica* E/S antigen levels in serum of experimentally infected sheep from the first week postinfection (wpi) and in fecal supernatant from the fourth wpi, which were significantly higher than controls, moreover *F.hepatica* eggs were not detected until eighth wpi.

Jefferies ***et al,*****(2001)** declare that it was possible to identify 29 of the 60 prominent proteins found in *F.hepatica* excretory secretory (ES) products using two-dimensional gel electrophoresis, results suggest that the relative abundance and protective nature of the components of the ES products of *F.hepatica* play an important role in its survival within the host.

Dixit ***et al,*** **(2002)** were used 28 KDa *Fasciola gigantica* cysteine proteinase in both ELISA assay and Westeren blot technique to detect *F.gigantica* antibodies, this was as a tool for diagnosis prepatent ovine fascioliosis.

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Reichel, (2002) tried to validate the ELISA for the detection of antibodies against liver fluke *(Fasciola hepatica)* in sheep and cattle sera, the results of performance of the ELISA demonstrate high sensitivity and specificity, moreover infection in experimentally infested animals could be discovered 7-8 weeks earlier than with classical parasitological techniques.

Paz-Silva *et al,* (2003) conclude that the combination of sandwish ELISA and coprological examination is extremely helpful for demonstrating current ovine fascioliosis, so application of sELISA is strongly recommended for epidemiological surveys.

Bernal *et al,* (2004) in order to identify proteins of *Fasciola hepatica* that could be involved in host-parasite interactions, by using different methods discover that, the enolase enzyme is present in the excretory-secretory materials of *F.hepatica*

2.3.1 Back ground on immune relationships between helminth parasites :

Rajasekariah *et al,(*1979) made a priliminary attemps in order to immunise rats and mice against infection wit *F.hepatica* by using *T.hydatigena* eggs and different antigenic prpartion, the reults showed that none of prepared antigens of *T.hydatigena eggs* were able to protect those animals against *F.hepatica* infection.

Campel *et al,* (1977) and Dineen *et al,* (1978) that the infection of sheep with *Taenia hydatigena* 12 weeks to nine months before infection with *F.hepatica* resulted in protection against infection *with F.hepatica*

El-Massry, (1988) stated that there is a vigorous immunological reaction to a helminth parasite can cause the simultaneous expulsion of other generically related and unrelated species even through it may not be essential for the target parasite to be antigenically related to the species used to promote the response, the perfect example of this fact is infection of sheep with *Cysticercus tenuicollis*, lambs which harbor this parasite can not be infected with *Fasciola spp.,* moreover perivous infection with

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C.teniucollis cause lowering infection rate with gastrointestinal nematodes or coccidia.

Warren, (1993) stated that, in between nematodes, especially of the same family sharing antigenic epitopes which may cause unspecific immune response to nematodal infection, and therefore declare that the sub optimal specificity observed in certain immunodiagnostic assays is primarily a consequence of sharing antigenic epitopes among nematodes parasites, shared epitopes clearly do exists in the surface and ES, so in recent years attentions turned to use of antigenic preparations which contain a more restricted range of epitopes, particularly those obtained from the parasite surface and/or ES.

2.3.2 Immune relationships between *Fasciola spp.* and other parasites:

Craig and Rickard, (1980) used a crude antigen prepared from the strobilate stage of *Taenia saginata* for the serological diagnosis of *T.saginata* cysticercosis in cattle using the enzyme-linked immunosorbent assay (ELISA), Sera were collected and tested from laboratory and pasture-reared calves experimentally infected with *T. saginata* as well as from cattle naturally infected by grazing on pasture irrigated with sewage effluent, the specificity of the "diagnostic" antigen was assessed using sera from laboratory-reared cattle with monospecific infections of *T. saginata, T.hydatigena*, *F.hepatica*, or gastro-intestinal nematodes, and natural infections of *F. hepatica* and observe that cross-reactions occurred in sera from all heterologous infections but the highest level occurred in cattle experimentally or naturally infected with *F. hepatica.*

Hillyer, (1980) after isolation of *Fasciola hepatica* tegumental antigens, and by using Ouchterlony immunodiffusion method proved potential of *F.hepatica* tegumental antigens for immunodiagnosis of rabbit and human fascioliasis, although it was noticed the presence of cross-reactivity between *Fasciola hepatica* and

Schistosoma mansoni, moreover he approved that *F.hepatica* tegument contained antigens can protect mice from challenge infection with *S.mansoni.*

Hillyer and Serrano, (1983) domenstrate the prescence of cross-reacting antigens btween *Paragonimus westermaani*,*Schistosoma mansoni* and *Fasciola hepatica* adult worms by using Ouchterlony immunodiffusion and enzyme linked immunosorbant assay (ELISA), indicating common antigenic determinants and suggesting a common antigen among digenitic trematodes.

Hanna and Hillyer, (1984) decided that there is an indication of existence of a common antigenic determinant between *Schistosoma mansoni*, *Paragonoimus westermani* whole worm extracts and *Fasciola hepatica* tegument antigens, indicate that cross-reactivity between tissue, stages, and species is due to the existence within these pools of common antigenic determents, also tried to identify the tissue sources of biochemically purified antigenic fractions of *Fasciola hepatica* and *Schistosoma mansoni,* by an indirect fluorescent-antibody-labeling technique, common antigens clearly originated from *F.hepatica* parenchyma, certain of these common antigens are known to provide significant protection in mice to challenge with *S.mansoni* cercariae, results indicate the presence common antigens in *S.mansoni* and *F.hepatica.*

Wang *et al*, (1985) in a study on ELISA for detection *Fasciola hepatica* in experimentally infected sheep, cross-reactivity was detected especially in the first 4 weeks p.i ; cross reactions between the sera of sheep infected with *F.hepatica* and sera of cattle with *Theileria annulata and Schistosoma japonicum* was observed, whereas there was no cross reactions with *Cyctericercus tenuicollis*, *Moniezia benedeni*, *Coenurus cerebralis*, *Echinococcus granulosus* and *Trypanosoma evansi.*

Klimenko and Belozerov, (1986) used *F.hepatica* proteinase as an antigen after purification in ELISA with sera from naturally infected sheep and experimentally infected sheep with *F.hepatica* results show that 22.4% of 58 control

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sera form uninfected animals or those with hydatiosis or *Cycticercus tenuicollis* react positively, but when sera from only the experimental animals were evaluated , one of 39 react positively.

Pathak *et al,* (1986) used counter immuno-electrophoresis in order to diagnosis of fascioliosis in sheep, from 10 blood samples from sheep infected with *F.hepatica*, 5 infected with *Cysteicercus tenuicollis* and 32 sheep free from parasites, results declare that there was no cross reactions between sera of experimentally infected sheep samples of *C.teniucollis* infected sheep and control sheep free from paras ites, and 9 sera samples from the 10 *F.hepatica* infected sheep gave positive reactions.

El-Massry, (1988) during examination of sheep slaughtered in Cairo abattoir observe that lambs which were infecting with *Cysticercus tenicollis*, did no show infection with *Fasciola*, i.e. infection with lamb with *Cysticercus tenicollis* prevent infection with *Fasciola spp.*, while only few percentage of examined animals (6.6%) all of them were aged females show mixed infection (*Cysticercus tenicollis* and *Fasciola*)

Santiago and Hillyer, (1988) tried to evaluate potential mechanisms of immunity in fascioliosis, so sera from sheep and cattles experimentally infected with *F.hepatica* were reacted with both somatic and excretory-secretory antigens, analysis of these sera by ELISA showed a significant increase in antibody levels by 2 wk in most infected cattle using both somatic and ES antigens, whereas with most infected sheep antibodies are not clearly detected until week 4, by using Enzyme Immunotransfer Blot (EITB) technique showed that both infected sheep and cattle recoconize major somatic polypeptides in a molecular weight range of 30-38 kDa by 8 wk, moreover cattle recognized 3 additional major antigens of 56,64 and 69 kDa as early as 6 wk, various polypeptides of 20-25 kDa are prominently detected by most sheep and very faintly, if at all, by cattles sera, also the sera from both sheep and cattles also identify ES polypeptides of 20-28 kDa, the patterns of polypeptides

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recognized by sheep infected with *S.mansoni* and challenged with *F.hepatica* are almost identical to those with a simple *F.hepatica* primary infection.

Hillyer *et al,* (1992) isolated *F.hepatica* glutathione S-transferase (FhGST) from adult worms, by SDS-PAGE showed 3 proteins of MW ranging from 29000-27800, by western immunoblot analysis revealed 2 bands in the same MW range, also mice and rabbits immunized with purified FhGST develop copious amounts of anti-FhGST antibodies; moreover, antisera *to F.hepatica* adult worms and excretion-secretion products also reacted with FhGST,also it was proved the presence of cross-reactivity of *F.hepatica* and schistomes in the reactivity with FHGST of anti-Schistosoma mansoni adult worm antisera and, to a lesser extent, antisera to *S.mansoni* soluble egg antigens, but must take in consideration that the time of appearance anti-FhGST antibodies in different species of animals infected with *F.hepatica* varies as in sheep and New Zealand white rabbit was 2 wk postexposure with *F.hepatica* and was detected by ELISA, while neither mice nor calves infected with *F.hepatica* developed antibodies to FhGST through 5-10 wk of infection tested, however mice infected with *S.mansoni* developed anti-FhGST cross-reacting antibodies by 6 weeks of infection, calves immunized with *Fasciola/Schistosma* cross-reactive, cross-protective antigen complex in which 12000 MW protein (Fh12) has been shown to contain immunoprophylactic activity, also developed antibodies to FhGST.

Arafa *et al,* (1999) tried to evaluate *Fasciola* E/S antigens for diagnosis of early fascioliasis using different immunological techniques such as Enzyme Immuno Blot Transfere (EITB) and Counter Immuno- Electrophoresis (CIEP), especially *Fasciola* specific E/S protein fraction (49.5kDa), observe that presence of cross-reactivity between *Fasciola* adult worm E/S products and *S.mansoni* .

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Rodriguez-Osorio *et al,* (1999) determined the circulating antigens, IgG antibody response to worm antigens and to excretory/secretory products (ES), and specificity to *Fasciola hepatica* antigens in 6 *schistosoma bovis* infected sheep results revealed tha E/s products was higher in respnce than somatic antigen, moreover observing that there was cross-reactivity between *Schistosoma bovis* and *Fasciola hepatica* antigens in sheep experimentally infected with *Schistosoma bovis,* they noticed a remarkable level of cross-reactivity when adult *F.hepatica* extract was used and a low degree of cross-reactivity by using ES antigens.

Mohey *et al,* (2000) through fractionation of whole-worm and mouth part antigens of *F.gigantica* and *T.vitulorum* by using SDS-PAGE showed obvious qualitative and quantitative differences, whereas three different anti-sera, raised in rabbits against adult extracts of *F.gigantica, T.vitulorum* and *Moneiza expensa,* were utilized in immunoblotting for identification of mouth part antigens that cross-react with adult worm of the same species or of different species, results revealed that 7 and 8 polypeptides were recognized in *F.gigantica* and *T.vitulorum* mouth parts respectively by their homologous rabbits anti-adult anti-sera, the cross reactive antigens in *F.gigantica* which recogonized by *T.vitulorum* anti-sera was 234 kDa, while two components of MW 113 and 93 kDa were detected by *M.expensa* serum in the same extract.

Abdel-Rahman *et al,* (2003) decided that by the aid of ELISA assay, there is cross-reaction between *Fasciola gigantica, Trichnilla spiralis* and *Echinococcus granulosus,* cross-binding activities in the prepared antisera were strongly directed towards protoscolices and hyadatid fluid antigens of *E.granulosus* rather than *F.gigantica* and *T.spiralis* antigens.

Romasanta *et al,* (2003) tried to identify the existence cross-reactivity among the excretory/secretory antigens of three important zoonotic parasites, *Fasciola hepatica, Toxocara canis* and *Ascaris suum,*and by the means of an indirect

ELISA observe that in experimentally infected rats IgM cross-reacting was mainly observed in first 10 weeks p.i.,and the IgG cross-reacting was observed throughout the study, and was maximal at the 2-3 weeks p.i.,which correspond the intraorganic migratory phase of these parasites; also the Western-blot showed that the rat IgG recognised three proteins of 190,160 and 33 kDa in the antigens of *F.hepatica, T.canis* and *A.suum.*

2.3.3 Immune relationships of *Cysticercus tenuicollis* with other parasites:

Kamaga-Sollo *et al,* (1987) used an antigenic fraction (ThFAS) isolated from *Taenia hydatigena* metacestode cyst fluid in an ELISA to detect antibodies to T.saginata in experimentally and naturally infected cattle, where in 10 calves given 1.000 to 100.000 *T.saginata* eggs (20% to 60% viability), IgG and IgM antibodies were detected in all calves by post-inoculation week 3, whereas IgG antibody values remained increased until calves were slaughtered at post-inoculation weeks 13 to 26, moreover 6 naturally infected calves (determined by postmortem examination) were considered positive, by using ELISA the shared antigens were demonstrated between ThFAS and *T.saginata* and *T.crassiceps*; there were no shared antigens between ThFAS and *Haemonchus contortus* or *Fasciola hepatica*, while immunoblot analysis indicated that a low molecular weight polypeptide (10.000 Mr) bears the immunodiagnostic antigen.

El-Massry, (1988) noticed that infection of sheep with *Cysticercus tenuicollis* (larval stage of *Taenia hydatigena*) may play another a role in protection of lambs against *Fasciola spp.* infection, moreover adult sheep which harbor *C.teniucollis* markedly decrease percentage of affection with *Fasciola spp.* infection, that observations suggest non-specific immuno-response of the host against *Fasciola spp.* and *C.teniucollis* infection.

El-Massry, (1999) serologically examined 500 sheep serum samples using ELISA, results revealed that 151 samples (30.2%) were seropositive for Taenia

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hydatigena metacestodes with optical density mean value 1.09+/-0.09, 0.56+/-0.09 and 0.31+/-0.04 for heavy, moderate and light infected animals respectively, regarding sensitivity of the test it was 91% that means out of 100 positive animals at postmortem (PM), only 91were seropositive with ELISA, regarding specificity it was 85% as 60 animals (15%) from 400 negative ones at P.M. showed positive results.

Panda ***et al,*** **(2000)** as a way for immunodiagnosis of ovine cysticercosis used a fraction of *Taenia hydatigena* metacestode, analysis of the fraction by sodium dodecyl sulphate polyacrylamide gel electrophoresis revealed the presence of 68 kDa protein, antibodies against the isolated protein were detected in 7 out of 10 experimentally infected lambs, further conformation of the diagnostic potential of the 68 kDa protein was done by testing sera from naturally infected post-mortem positive (PM+) and from apparently healthy groups of animals, 80% an 8% of animals were found positive by ELISA in the groups of PM+ and apparently non0infected lambs, respectively.

Kara ***et al,*****(2003)** by using Western blotting technique revealed the protein bands in cyst fluid antigen of *C.teniucollis* also immune reactive bands were determined, by using 10 positive and 10 negative sera for *C.teniucollis* from sheep and one non-infected sheep serum were tested, results showed that there was only one protein band determined to be immune reactive, which was 36 kDa.

2.3.4 Immune relationships of abomasal nematodes with other parasites:

Keus ***et al,*** **(1981)** found that the ELISA assay is very useful for the detection and monitoring of anti-*Cooperia* and anti-*Ostertagia* antibodies in calves, using six different antigens saline extract from third- and fourth-stage larvae and from adult worms of both genera; results revealed that some degree of genus specificity was found when using L4 or adult worm antigens but when L3antigens were used.

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Kloosterman ***et al,*** **(1984)** studied the interactions between *Ostertagia ostertagi* and *Cooperia oncophora* in calves by concurrent and sequential infections, they observe the presence of a reciprocal negative interaction between the species in sequential, but not in concurrent infections, this result was supported by the finding of serological cross-reaction.

Miller, (1984) stated that the application of immunochemical and molecular techniques has enabled much more prices antigen analysis to be undertaken and has provided the means of studying immune responses to precisely defined molecules, among the most useful of these techniques have been surface and metabolic labeling and ELISA.

Canals and Gasbrarre,(1990) tried to isolate and charachterise of two somatic antigens (Adult worm somatic extract and fourth/fifth molt extract), and an metabolic antigen (E/S antigen) of *Ostertagia ostertagi*; they observe that presence of strong cross-reactivity in the ELISA prepared one metabolic (ES) and two somatic extract (AS and MS) from *Ostertagia ostertagi*, and by using SDS-PAGE and immunoblot techniques partial characterization of theses extract was done, in immunoblots, AS showed a low number of bands that reacted weakly with sera collected from infected calves, while MS reacted strongly with homologous sera, moreover a sharp group of bands appeared from 12 to 14 kDa, increasing intensity as the infection progressed, regarding ES, it showed a group of strongly immunogenic bands in the range of 16-22 kDa, however an ELISA was used for detection the reactivity of the three extracts with specific anti-Ostertagia antibodies, and ELISA results showed that all three worm extracts contained antigen epitopes recognized by circulating antibodies in sera taken from *O.ostertagi* infected calves, but the strongest response was seen when antibodies of IgG1 isotype were reacted with MS and ES, also it noticed that when sera taken from *O.radiatum*-infected calves were used both somatic extracts showed high levels of cross-reactivity.

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Hendrikx, (1990) examined various tissues (the following tissues containing antigens: the sheath and cuticle of L3 larvae; and the cuticle, muscles, epithelium and glandular tissue of the oesophagus and sheath of the spicules of adult nematodes) of L3 larvae and adult *Haemonchus contortus* by indirect fluorescence and electrophoresis to investigate antigenicity, cross-reactions occurred with antigens in the L3 fractions that were not injected into the rabbits, some of the cross-reaction was caused by antibodies were induced against phosphorylcholine.

Cuquerella *et al,* (1993) made infection of 16-18 weeks old lambs with 2500 3rd stage larvae (L3) of *Haemonchus contortus* or kept as uninfected controls, 2 months later all animals were challenged with 5000 L3 of the same parasite, while soluble antigens of *H.contortus* L3 and adult worms were analyzed results showed that antigens from both sources, particularly of low molecular weight were recognized by the pooled sera of infected lambs, moreover no single L3 antigens was recognized by all infected lams, whereas 2 peptides having around 25 and 26 KDa from adults were recognized by infected animals during the patency and could be a potential use in the diagnosis of lamb haemonchosis.

Cuquerella *et al,* (1994) and as a preliminary trial on the extend of cross-antigenicity among the sheep strongyloids *Haemonchus contortus, Trichostrongylus columbriformis, Teladorsagia circumcincta* and *Nematodirus battus* in 2.5 to 4-months lambs, ELISA and Western blotting (WB) have been carried out, cross reactivity was tested using soluble extracts from adult and third stage larvae (L3) of *Haemonchus contortus* as antigenic source probed with sera from lambs with monospecific heterologus infections, results revealed that there was cross-antigenicity between L3 of *H.contortus* and *T.Colubriformis* in ELISA and WB, while immunodetection results with adult *H.contortus* antigens showed a closer relationship to *Teladorsagia circumcincta,* also it was observed that certain heterologous sera reacted with *H.contortus* antigens more strongly than the homologues ones, but sera from *H.contortus* infected animals had reactivity around the 25 kDa region from adult antigens which could have potential diagnostic use.

Schallig ***et al,*** **(1994)** observed during their study on Immune responses of Texel sheep to excretory/secretory products of adult *Haemonchus contortus*, the excretory/secretory (E/S) products of adult *Haemonchus contortus* comprise of at least 15 polypeptides with molecular weights ranging from 10 to > 100 kDa, some of E/S products *of H.contortus* showed immunoreactivity with serum samples of *Haemonchus*-infected sheep as well as with samples of sheep harbouring other *Trichostrongylid* infections; these cross-reacting epitopes are the main cause of the lack of specificity of an E/S material-based ELISA, so ELISA can differentiate *Haemonchus infections* from *Nematodirus battus* infections, but not from *Ostertagia circumcincta* or *Trichostrongylus colubriformis* infections.

Schallig ***et al,*** **(1995)** conclude that under the field conditions when infections with gastrointestinal helminthes and *Eimeria spp* is the rule, this will limits to detect *H.contortus* infection in sheep by using ELISA either using excretory/secretory (ES) products or Crude somatic antigens (CSA) of adult *Haemonchus contortus* in two enzyme immunosorbent assay.

Gomez-Munoz ***et al,*** **(1996)** by means of gel filtration (S-200) and anion exchange chromatography (DEAE-Sephacel), the adult *Haemonchus contortus* soluble extracts were fractionated, and fractions from both analyses were checked by ELISA and Western blotting (WB) with sera from lambs infected with *H.contortus*, monospecific heterologus sera (anti-*Trichostrongylus columbriformis* and ant-*Telasdorsagia circumcincta*) and sera from naturally infected sheep with mixed trichostrongylid infection, when fractions from gel filtration were checked by ELISA found that there was high cross reactivity between *H.contortus* and heterologous sera, particularly with the anti-*T.columbriformis* serum, it seems that 26 kDa antigen is apparently specific for the diagnosis of *H.contortus* infections in lambs.

Molina ***et al,*****(1999)** as a method for detection of cross-reactive antigens of *Haemonchus contortus* adult worms in *Telasdorsagia circumcincta* infected goats, the adult *Haemonchus contortus* somatic antigens responcible for cross-reactivity

have been analyzed using serum samples from goats kids infected and re-infected with *Telasdorsagia circumcincta*, results showed that goats kids infected with *T.circumcincta* had similar serum ELISA values against somatic antigens of *H.contortus* as goats infected with *H.contortus* itself, by using immunoblotting this extensive cross-reactivity was confirmed particularly in the molecular weight range 105-29 kDa, but peptides with high (195,152 and 119 kDa) or low (23 kDa) molecular weight were only faintly recognized by heterologus sera.

Gomez-Munoz *et al,* (2000) collected sera from 53 sheep belonging to Castellano, Churro, Manchego, and Merino breeds and these collected sera were analyzed to test the diagnostic value of 26-KDa antigen from adult *Haemonchus contortus* at prepatency and early and late patency of experimental haemonchosis , in which animals that received zero, 1, or 2 infections with the parasite were tested, in addition sera from 20 experimentally infected and 10 noninfected Texel sheep were used to test, results showed that primary and secondary infection were found positive with the 26-KDa antigen, however sera from 10 animals with the lowest worm burdens (second infection) did not recognize the antigen during early patency (day 28 postinfection), moreover the antigen specificity was confirmed because hyperimmune sera against infective larvae and adult stages of the most common gastrointestinal nematodes found in natural infections in sheep (*Trichostrongylus columbriformis* and *Teladorsagia circumcincta*) did not recognize this peptide, while this antigen was recognized only by anti-adult *H.contortus* hyperimmune sera and appeared to be absent in the L3 parasitic stage, in addition, the partial N-terminal amino acid sequence of the diagnostic peptide is reported.

Derbala and El-Rahman, (2001) on 100 fecal specimens and corresponding blood samples and for evaluation of *Haemonchus contortus* infection in sheep by using ELISA assay with somatic, circulating and coproantigens, results proved that coproantigen was more potent than the others in diagnosis of sheep haemonchosis, the potency of coproantigen may be attributed partially to one polypeptide of

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molecular weight 45 kDa, this antigen was expressed as an immunogen which probably increase the immunogenicity, and may have potential and protective values.

Knox and Smith,(2001) showed that the proteins isolated from the surface of the gut of gastrointestinal nematodes, particularly *Haemonchus contortus*, have generally proved to be useful protective antigens and several are being progressed towards recombinant protein-based vaccines, some of the most promising antigens include contortin,H11, H-gal-GP, GPI and cysteine proteinases.

De Maere *et al*, (2002) in order to identify *Ostertagia ostertagi* antigens that were exclusively recognized by antibodies from immunized calves, used Western blots of extracts and excretion/secretion (E/S) material from L3, L4 and adult life-stages, results show that in the adult stage, a protein of 32 kDa was specifically detected on Western blot by mucus antibodies from immunized animals, while in the L3 and L4 larval stages proteins situated in the region of 28-29 kDa that were recognized by mucus antibodies and a 59 kDa antigen was specifically recognized by lymph node antibodies from immunized animals.

2.5 Histopathological studies:

Ibrahim, (2002) stated that livers infected with *F.gigantica* microscopically charachterised by biliary fibrosis, fibrous tissues formation around bile duct contain mature flukes, other ducts showed hyperplasia.

El-Dakhly, (2003) observe several microscopical changes in liver of non-immunized sheep infected with *Fasciola gigantica.*, these changes includes formation of many accessory bile ducts, trauma and necrosis of hyperplastic mucosa, fibrosis and irregular infiltration of esinophils, plasma cells and lymphocytes.

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III-Material and Methods

1-Collection of samples:

A-Collection of different helminthes and the related sera samples:

One hundered and eighty nine sheep were examined at aperiod extended from January 2005 till December 2005 during P.M. examination in El-Mahalla Kubra abattoir for presence of different helminthes with special attention for *Fasciola spp.*, adult cestodes, metacestodes (*C.teniucollis*) and abomasal nematodes. The recovered worms were collected from naturally infected sheep according to **Pritchard and Kurse , (1982)**.

During the slaughtering and bleeding of the sheep, one hundered well marked blood samples were collected in serially labeled screw 15 ml capacity plastic tubes, then placed at angle 45 and transferred to the laboratory of parasitology department in faculty of Veterinary Medicine at Kafr El-Sheikh, where these labeled blood samples were centrifuged at 3000 rpm/5 minutes in order to obtain clear labeled sera samples related to the slaughtered sheep number and the collected worms, then labeled sera were preserved at –20 °C until use.

The microscopical study of the collected worms was done according to **Abdel Rahman *et al, (*1982)** and **Fleck and Moody, (1993).**

Fasciola spp. were collected through examination and exposure of bile ducts of infected liver, then placed in Pettri dish and washed several times with distal water to remove the tissue debris, blood and contaminants, thenafter several times by using normal saline, lastely by using PBS.

Whereas, the collected adult cestodes were washed with tape water to remove the gross contamination, then thoroughly with distal water, then kept in normal saline, some of it were preserved in formalin solution 10%, others were examined and

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prepared to be permanently fixed and stained and some of the collected adult cestode worms placed on glass slides in order to identify the species.

While, metacestodes (*C.teniucollis*) which collected were washed several times with distal water then, the host tissue debris were removed by using scissor and forceps, rewashed by using normal saline, cysts were examined under stereo microscope for viability after placing it in warm distal water, finally some of these cysts preserved in formalin solution 10%.

For recovery of abomasal worms, abomasa of slaughtered sheep were collected unopened and transferred to the laboratory where there were opened and the contents were partilay placed in several wide Pettri dishes and add about 50 ml of warm normal saline on each dish, while the abomasa were scraped, washed and examined for the presence of adult nematodes by aid of naked eye, hand lens and stereo microscope, the adult nematodes were picked up by using fine forceps and transferred into another clean Pettri dish filled with normal saline this was done according to **Pritchard and Kurse, (1982)** and **Solusby, (1982)**, some of these adult nematodes were preserved in glycerol alcohol, other were fixed on glass slides for determine the species.

2- Preparation of different antigens from the collected worms :

1- Preparation of crude *Fasciola spp.* antigen (CFA):

The crude *Fasciola spp.* antigen was prepared according to **Mousa, (1992)** as following steps adult, *Fasciola* worms were collected from infected liver from the abattoir, the collected worms were washed several times with PBS, PH 7.4 for about 1 hour, then homogenized thoroughly with attention that during homogenization the glass tube must kept in ice, kept vertically and moved up and down several times, after homogenization the homogenate were sonicated, then centrifuged and the supernatant now consider as CFA, ampulated in Eppendroff's tubes (2 ml capacity) and stored at –20 °C until used.

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2- Preparation of excretory/secretory (E/S) antigen of *Fasciola spp.*

The E/S products were prepared according to **Mousa, (1992)** as follows, After obtaining adult worms from the abattoir, the collected worms were washed repeatedly in PBS, PH 7.4 then incubated for 3-12 hours in PBS, then remove the worms and collect the supernatant which centrifuged and ampulated in Eppendroff's tubes (2 ml capacity) and stored at –20 °C until used

3- Preparation of different antigens of *Cysticercus tenicollis:*

A- Preparation of whole cyst antigen of *Cysticercus tenicollis*

C.teniucollis whole cyst antigen was prepared according to **El-Massry, (1988)** as follows, The freshly obtained cysticerci from the abattoir, host tissues were removed by a scissor with great care, then washed repeatedly in double distilled water and kept overnight at 4 °C, then the cysts were opened and their fluids was added to the cysts, homogenization was done after chilling in ice and then centrifuged at 5000 rpm/ 30 min, obtain the supernatant and recentrifugation occurs for 2-3 times until no sediments was thrown down, ampulated in Eppendroff's tubes (2 ml capacity) and stored at –20 °C until used

B-Preparation of cystic fluid antigen of *Cysticercus tenicollis*

C.tenicollis cystic fluid antigen was prepared according to **El-Massry, (1988)** as following the freshly obtained cysticerci from the abattoir, host tissues were removed by a scissor with great care, then were washed repeatedly in double distilled water, the cystic fluid were collected with the help of sterile syringe, then the collected fluid was ampulated in Eppendroff's tubes (2 ml capacity) and stored at –20 °C until used

4- Preparation of crude antigen of adult *Haemonchus spp.*

Adult *Haemonchus spp.* whole worm antigen was prepared according to **Kandil, (1994)** with some modifications, as following, after collection of adult worms, they

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were washed several times with PBS, chopped into short pieces and homogenized, centrifugated at 5,000 rpm / 30min, then collect the supernatant and ampulated in Eppendroff's tubes (2 ml capacity) and stored at –20 °C until used.

3-Determination of total protein content in different antigens preparation:

After preparation of different antigens from different collected helminthes, the protein content of each was determined according to modified **Lowry's assay, (1951)** as following:

A- Preparation of the test standard curve:

Standard protein; bovine serum albumin (BSA) solution was prepared (standard curve) between 0 and 100 µg/ 200µl/PBS by using different concentration of BSA in PBS within 9-glass test tubes

No. of test tube	BSA in µl	PBS in µl	Protein content
1	-	1000	0
2	50	950	10
3	100	900	20
4	150	850	30
5	200	800	40
6	250	750	50
7	300	700	60
8	400	600	80
9	500	500	100

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B- Procedures:

A series dilutions of the unknown protein solution was prepared, started at 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 200 µl was added into glass tubes which contain buffer used to dissolve the unknown protein.

0.5% copper sulphate in 1% sodium tartrate was prepared by mixing equal volumes of copper sulphate and sodium potassium tartrate solution.

1 ml is removed and mixed with 50 ml of 0.2% sodium carbonate in 0.1% sodium hydroxide, this preparation is freshly prpared for each assay.

1 ml of this solution was added to each of the tube containing the standard and the unknown protein solution, mixed well,each tube mixed and allowed to stand for 20 minutes at room temperature.

100 µl of Folin and Ciocateau's reagent were added to each tube and shacked vigorously.

Incubate the tubes at room temperature for 30 minutes.

The colour reaction was quantitate in a spectrophotometer at 500 nm.

Absorbance against protein concentration of the standard solution was plotted, the protein concentration equivalent to the colour reaction of the unknown was determined (OMP).

Finally, the protein concentration was calculated using the following equation:

O.D. of sample/ O.D of standard **X** Conc. Of Standard **X** 5 **X** final sample solution

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4- Application of Enzyme Linked ImmunoSorbent Assay (ELISA) according to Craig and Rickard, (1980), Iacona *et al*, (1980) and El-Dakhly,(2003) :

Checkerboard Titration:

Checkerboard titration was done in order to figure the optimum dilutions of both serum and antigen, with regard that know dilution of conjucate and substrate is used, therefore known positive pooled serum were diluted as 1/100, 1/200, 1/400, 1/800 and 1/1600, also the antigen was diluted as 5 µg/ml coating buffer,10 µg/ml coating buffer, 20 µg/ml coating buffer , 40 µg/ml coating buffer and 80 µg/ml coating buffer, then the test procedures was performed as usual regarding that the first serum dilution react with the lowest dilution of antigen (showed as obivious difference in O.D. even by naked eye) considered the best dilutiuon of both serum and antigen to be used and applied in further tests performed by the same antigen and unknown sera.

1)-Reagents:

1) 96 well (8 longitudinal strips each contain 12 well) flat bottom microculture polystyrene plates.
2) Washing buffer: (PBS-T 0.05%) Phosphate Buffer Saline (PBS) containing 0.05% Tween-20.
3) Coating buffer: 0.1 M sodium carbonate Ph 9.6.
4) Blocking Buffer: Coating buffer containing 0.1% bovine serum albumin.
5) Diluting buffer: 0.01 M PBS.
6) Conjugate: Anti-sheep IgG (whole molecule) Alkaline Phosphatase, produced by Sigma® (used as instruction of the manufacture).
7) Substrate buffer: 0.05 M sodium carbonate, Ph 9.8 containing 0.001 M Magnesium chloride

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8) Substrate: P- nitrophenyl phosphate (used as instruction of the manufacture).

9) Sera: which collected from naturally infected sheep, with control –ve ones

10) Antigens: different antigenic preparations of (*Haemonchus spp.* and *C. tenuicollis* whole cyst antigen) were used after checkerboard titration.

Procedures:

1) Antigen diluted in coating buffer at their optimal concentration which was determined after preparation of different antigen concentration and different dilution of sera.

The optimal dilution rate was 40 µg/ml coating buffer.

2) Each well was filled with 100 µl of the corresponding antigen concentration.

*Wells were incubated overnight at room temperature.

3) *Wells were washed 3 times with PBS-T 0.05%.

*Blocking of the remaining free binding sites with blocking buffer by addition of 200 µl/ well/ incubated for 2 hrs/ at 37 °C.

4) *Wells were washed 3 times with PBS-T 0.05%.

5) *Different sera diluted (in ratio 1: 100) in PBS, Ph 7.4 were added 100 µl/ well/ incubated for 2 hrs/ at 37 °C.

6) *wells were washed 5times with PBS-T 0.05%.

7) * Conjugate was added in dilution (1: 1000 in PBS, Ph 7.4), added by rate 100 µl/ well/ incubated for 1 hrs/ at 37 °C.

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8) *wells were washed 5times with PBS-T 0.05%.

*wells were washed twice with substrate buffer.

*Substrate was added (1 mg/ 1 ml of P-nitrophenyl Phosphate) 50 µl/ well/ incubated for 30 minutes/ at 37 °C.

9) * Reaction appear with yellow coloration

* reaction was stopped by addition of 1N NaoH 50 µl/ well.

10) *Measuring was done on ELISA reader (star Fax 303+, 12 well strips ,1-12 at absorbance 405 nm).

V- Histopathological examination:

Liver which was collected from naturally infected sheep during P.M. examination was cut into fine pieces, fixed in 10% formalin solution and embedded in paraffin-sections 5-7 microns in thickness, then stained with Haematoxylin and Eosin stain (H&E) according to **Carlton (1967) and Lillie *et al* (1976).**

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IV- Results

A)- Incidence of different helminthes of sheep:

One hundred eighty nine sheep of different breeds, sex and ages were examined during post-mortum examination in El-Mahalla Kubra abattoir for presence of different helminthes with special attention for *Fasciola spp.*, adult cestodes, metacestodes (*C.teniucollis*) and abomasal nematodes.

Results showed that 98 (51.9%) sheep were infected with helminthes, sheep harbouring trematodes were only 5 (2.65%), while those harbouring adult cestodes were 39 (20.64%), sheep infected with metacestodes (*C.teniucollis*) were 32 (16.93%) and the animals harbouring abomasal nematodes were 22 (11.64%). [Tables (1) & (2) and Plates (I) & (II)].

Fasciola gigantica was recovered in only one sheep (0.53%), *Paramphistomum cervi* was in only 4 sheep (2.12%), *Moneizia expensa* was the most recovered spp. of adult cestodes from the examined sheep, 36 (19.04%) also, *A vitellina centripunctata* as adult cestode was recovered but in only 3 cases (1.6%), whereas *Haemonchus contortus* was the most predominant recovered nematode species from abomasa of 15 sheep (7.9%), other nematodes species i.e. *Graphidiops spp and Parabonema skrjabini* recovered from only in 7 (3.7%). [Plate III]

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Table (1): General incidence of infection with different helminthe parasites in examined sheep:

Examined animals	Infected No.	%
189	**98**	**51.9%**

Table (2) Incidence of infection with different helminthe species in examined sheep:

Category	Species	No. of infected	%
Trematodes	***F.gigantica***	**1**	**0.53**
	P.cervi	4	2.12%
Cestodes	***M.expensa***	**36**	**19.04%**
	A.centripuctata	3	1.6%
Metacestodes	***C.teniucollus***	**32**	**16.93%**
Nematodes	***H.contortus***	**15**	**7.9%**
	Graphidiops spp & Parabonema skrjabini	7	3.7%

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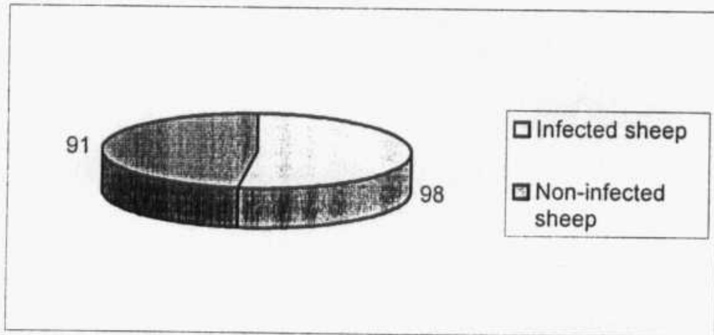

Plate (I): Incidence of infection with different helminthes in examined sheep.

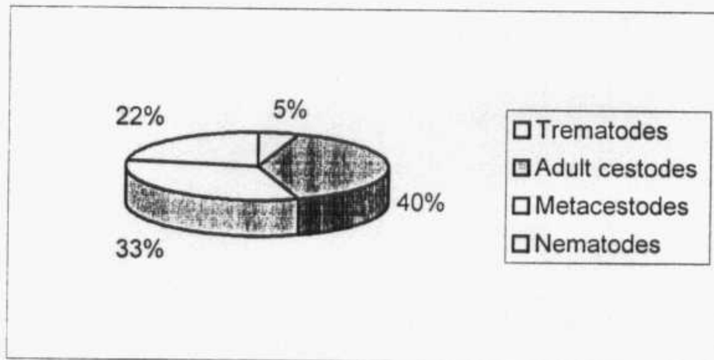

Plate (II):Incidence of different helminthes in examined sheep.

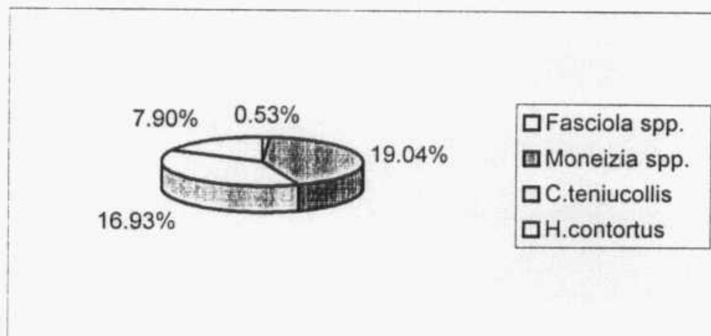

Plate (III): Incidence of helminthes species in examined sheep.

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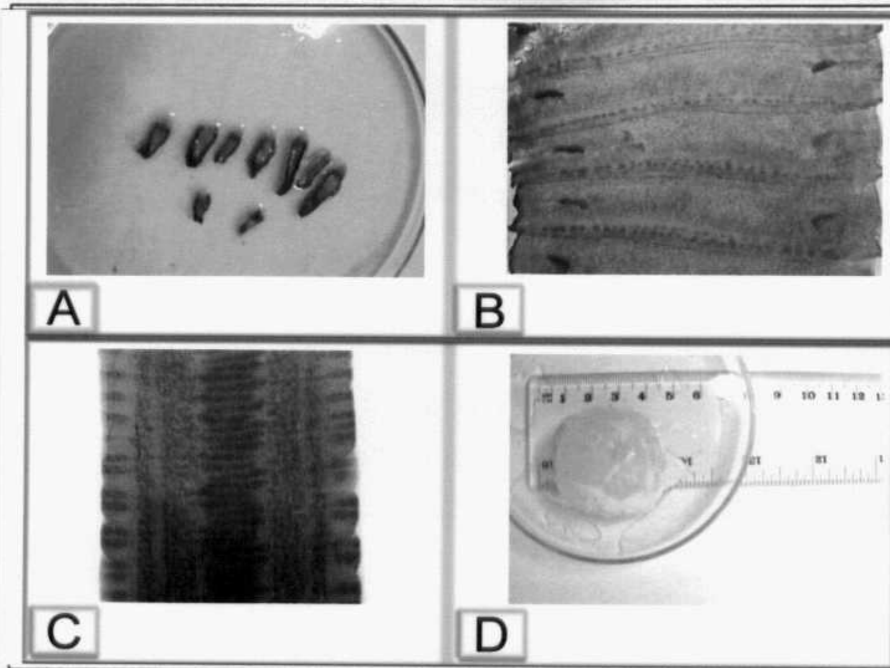

Plate (IV):

A.)*Fasciola gigantica* in Petri dish.

B.) *Moneizia expensa* mature segment

C.)*Avitellina centripunctata* mature segment

D.)*Cysticercus teniucollis* in Petri dish.

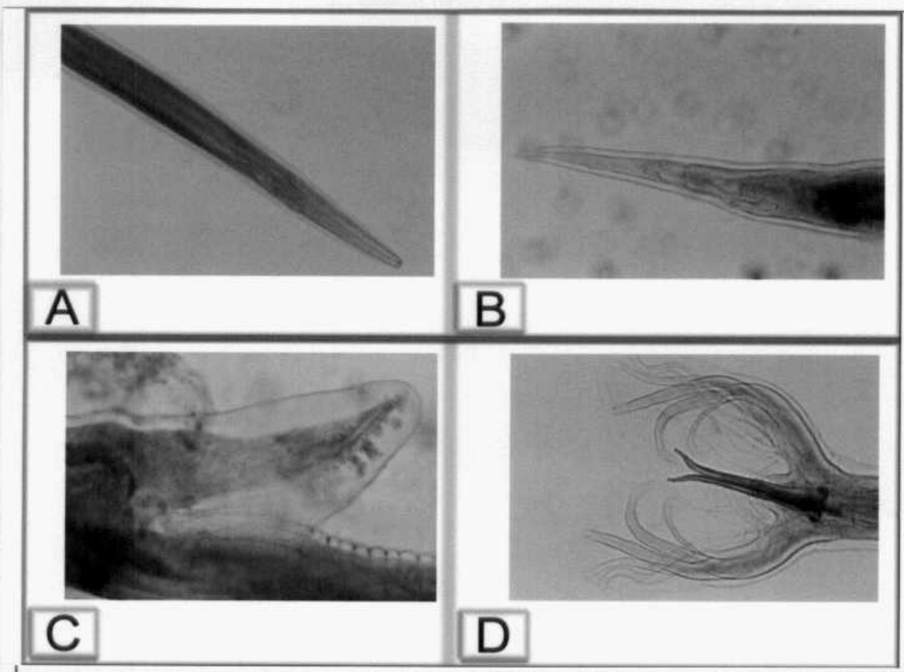

Plate (V):

A.)*H.contortus* (Ant.End X10).

B.) *H.contortus* (Femlae Post. End X40)

C.) *H.contortus* (Femlae vulvular flap X40)

D.) *H.contortus* (Male copulatory bursaX40)

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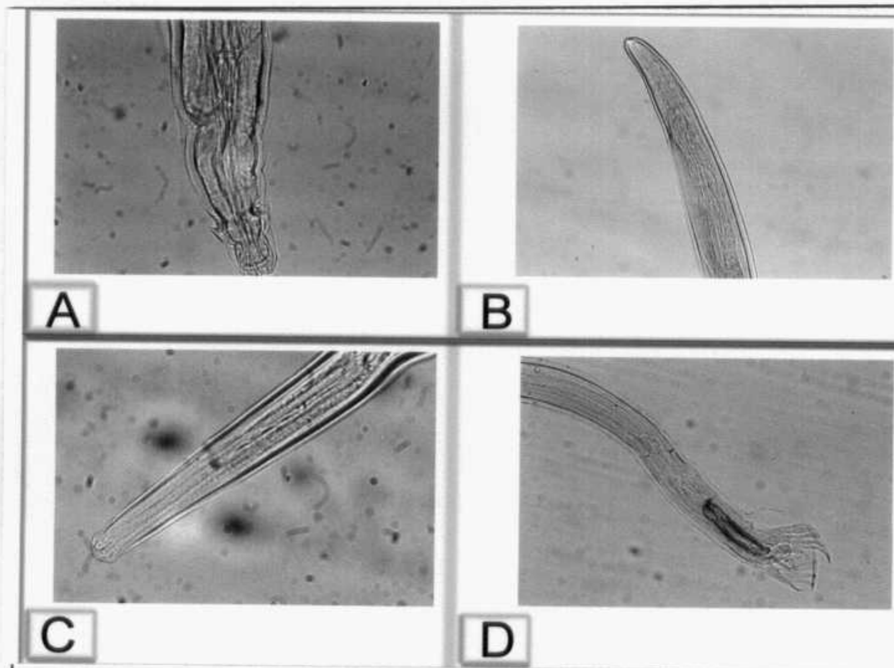

Plate (VI):

A.)*Parobronema skrajbini* (Ant.End X40).   B.) *Parobronema skrajbini* (Femlae Post. End X10)

C.) *Graphidiopis spp.*(Ant. End. X40)   D) *Graphidiopis spp.* (Male copulatory bursaX10)

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Regarding the incidence of mixed infection with different helminthes in sheep, it was found that the mixed infection among examined sheep, 24 (12.698%) were harbouring mixed infection by more one species of helminthes.

The most common mixed infection was mixed infection with *Haemonchus contortus* and other species of abomasal nematodes i.e. *Graphidiops spp and Parabonema skrjabini*, 10 (41.7%) out of 24 cases were recorded.

While mixed infection with *Moneizia expensa* and *C.teniucollis* was 7 (29.16%) out of 24 cases, while mixed infection with *A vitellina centripunctata* and *C.teniucollis* was only 2 (8.33%) cases out of 24, however other mixed infection such *F.gigantica* with *Haemonchus contortus*, *Moneizia expensa* with *A vitellina centripunctata*, *Moneizia expensa* with *Haemonchus contortus*, *Moneizia expensa* with other species of abomasal nematodes i.e. *Graphidiops spp & Parabonema skrjabini* and finally *C.teniucollis* with *Haemonchus spp.* were recorded and each was observed in a single case (4.16%) out of 24 sheep had a mixed infection only by incidence of infection for each type of mixed infection. [Table 3 and Plate VII]

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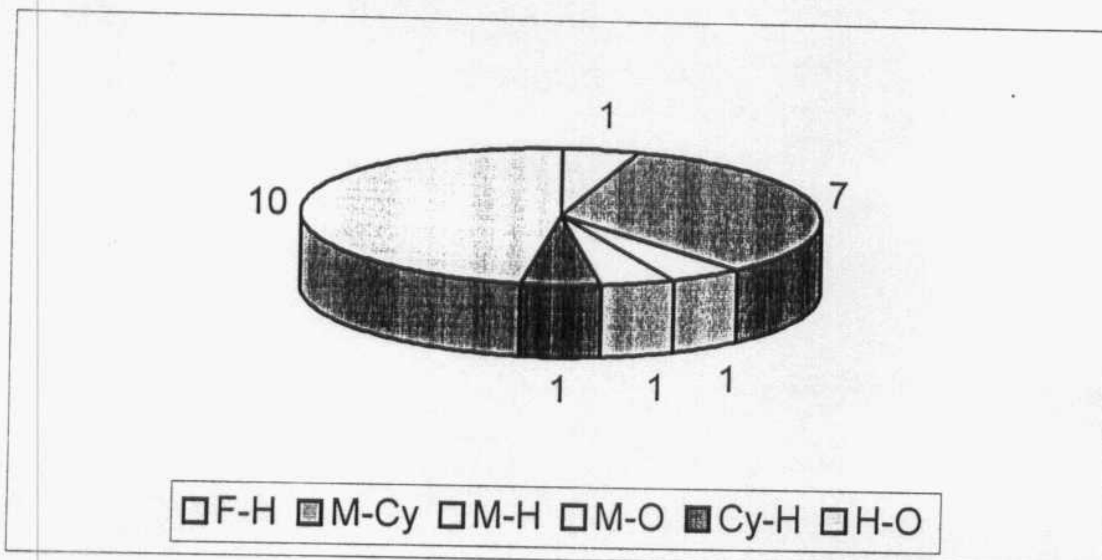
Table (3): Incidence of mixed infection in Examined sheep

No. of infected animals	Type of Mixed Infection	%
1	*F.gigantica* and *H. contortus.*	4.16%
1	*Moneizia spp.* and *A.centripunctata*	4.16%
7	*M.expensa* and *C.teniucollis*	29.16%
1	*M. expensa* and *H.contortus*	4.16%
1	*M.expensa* and *Parobronema spp. & Graphidiops spp.*	4.16%
2	*A.centripunctata* and *H.contortus*	8.33%
1	*C.teniucollis* and *H.contortus*	4.16%
10	*H.contortus* and *Parobronema spp.& Graphidiops spp*	41.7%

Plate (VII): Incidence of mixed infection in examined sheep:



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C)- Application of ELISA assay to determine the sensitivity of different antigens:

After checkerboard titration of different antigenic preparation (*Haemonchus contortus* crude antigen *C.teniucollis* whole cyst antigen and sera has been performed, the indirect ELISA procedures were performed, after ELISA procedures were applied readings were obtained by ELISA reader (star Fax 303+, 12 wells, 1-12 at absorbance 405 nm).

1- Determination of sensitivity and specificity of *H.contortus* crude antigen for diagnosis of *H.contortus* infection in sheep by ELISA:

Different sera collected from apparently non-infected and naturally infected sheep used at an optimal dilution 1:100 were tested by ELISA in order to figure the sensitivity and specificity of prepared antigen which used at its optimal concentration (40 µg/ ml coating buffer).

The cutoff value (which calculated as double fold of mean of the negative sera, the negative sera that were used derived from apparently non-infected sheep) was 0.326.

Seven sera out of eight derived from naturally infected sheep that were harbouring *H.contortus* infection gave positive reaction, so the sensitivity was 87.5%. [Table (4)]

2- Determination of sensitivity and specificity of *C.teniucollis* whole cyst antigen for diagnosis of *C.teniucollis* infection in sheep by ELISA:

Different sera collected from apparently non-infected and naturally infected sheep used at an optimal dilution 1:100 were tested by ELISA in order to figure the sensitivity and specificity of prepared antigen which used at its optimal concentration (40 µg/ ml coating buffer)., The cutoff value was 0.293.

Sera derived from 27 out of 30 naturally infected sheep that were harbouring *C.teniucollis* infection gave positive reaction, so the sensitivity was 90%. [Table (5)]

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O.D. readings											Control –ve
0.187	0.312	0.253	0.173	0.211	0.249	0.395	0.121	0.207	0.146	0.254	0.146
0.223	0.175	0.225	0.201	0.140	0.247	0.265	0.361	0.219	0.214	0.366	
0.253	0.229	0.175	0.216	0.214	0.390	0.258	0.207	0.225	0.175	0.389	0.197
0.203	0.121	0.210	0.195	0.225	0.253	0.226	0.388	0.317	0.224	0.214	
0.219	0.181	0.296	0.212	0.359	0.153	0.235	0.149	0.308	0.178	0.290	0.180
0.189	0.267	0.192	0.292	0.310	0.314	0.269	0.310	0.246	0.214	0.270	
0.184	0.237	0.294	0.185	0.214	0.202	0.260	0.223	0265	0.298	0.240	0.130
0.185	0.210	0.214	0.240	0.145	0.147	0.201	0.215	0.145	0.200	0.122	

Table (4): Determination of the sensitivity of *Haemonchus contortus* crude somatic antigen by using ELISA assay.

*** Cut off value = 0.326**

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O.D. readings											Control –ve
0.302	0.313	0.295	0.299	0.330	**0.198**	**0.201**	**.0250**	0.295	**0.156**	0.299	**0.145**
0.210	**.0145**	**0.190**	**0.210**	**0.197**	0.329	**0.218**	0.330	**0.185**	**0.201**	**0.189**	
0.240	**0.129**	**0.158**	**0.190**	**0.210**	**0.240**	**0.189**	0.310	0.348	**0.175**	**0.210**	**0.169**
0.130	**0.158**	**0.210**	**0.150**	**0.120**	**0.200**	**0.210**	0.300	0.310	**.0247**	**.0120**	
0.280	**0.180**	0. 350	**0.160**	**0.175**	**0.153**	**0.210**	**0.174**	0.298	**0.218**	**0.167**	**0.125**
0.277	**0.223**	**0.180**	0.366	**0.189**	0.321	**0.158**	0.302	**0.124**	**0.165**	**0.156**	
.0.201	**0.224**	**0.228**	0.350	0.298	**0.218**	0.303	0.313	0.294	0.299	0.314	
0.315	**0.198**	**0.225**	**0.165**	**0.201**	**0.128**	**0.285**	**0148**	**0.189**	**0.175**	**0.215**	

Table (5): Determination of the sensitivity of *Cysticercus tenuicollis* whole cyst antigen by using ELISA assay.

*** Cut off value = 0.293**

D)-Results of histopathology:

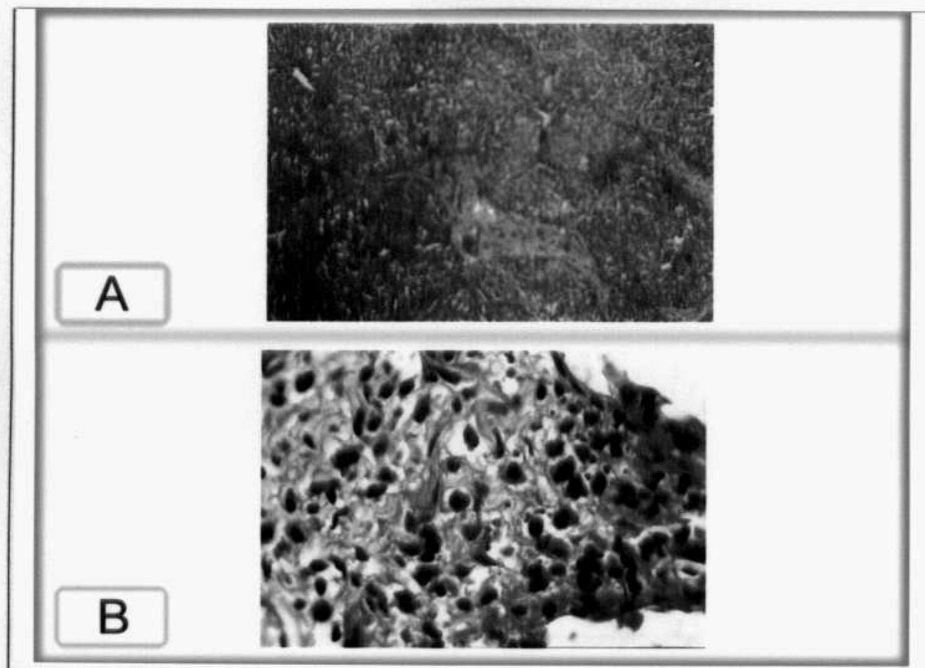
Results indicated that liver from the infected sheep showing fibrosis, periductal hyperplasia, and many accessory ducts formed and leukocytic infiltration by lymphocytes.

Heavy leukocytic infiltration was observed and most of the infiltrated cells were esinophils and macrophages.

Plate (VII):

A.) Liver of sheep infected with *Fasciola gigantica* showing sever fibrosis.(H&E)

B.) The same liver tissue showing heavy leukocytic infiltration, especially macrophages and esinophils.



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V- Discussion

In the present study, gross examination of 189 slaughtered sheep of different ages and sex was performed.

The results indicated that 98 (51.9%) out of 189 sheep had an infection with helminth parasite, these results were lower than results obtained by **Kedees, (1990)** whose results indicated that 22 (64.7%) out of 34 sheep were infected with helminth parasites, also were lower than results obtained by **Abd El-Miguid, (1998)** whose results indicated that 144 (72%) out of 200 intestinal tracts of sheep had different helminthes, but these results were higher than results obtained by **Khalafallah, (2000)** whose results indicated that the incidence rate of different helminthes was out 78 (45.1%) out of 173 examined sheep, in author opinion the prevalence rate of different helminthes infection among examined sheep considers high rate, as almost half of the examined sheep had helminth infection, this may be attributed to the feeding behavior of those sheep and mangemental factors during grazing.

While 24 (12.7%) out of 198 examined sheep had a mixed infection with more than one species of helminthes, this result was lower than of **Abd El-Miguid, (1998)** whose results indicated that 64 (32%) out of 200 sheep had a mixed infection with more than one species of helminth parasites, but this result was higher than result obtained by **Khalafallah, (2000)** who result indicated that 19 (11%) out of 173 sheep had a mixed infection with helminthes, this may be due to the mangemental factors during grazing also.

The prevalence rate of *F.gigantica* in examined sheep was 1 (0.53%) out of 173 examined sheep, this result was lower than this of **El-Sayed, (1991)** who used both coprological examination which revealed that 311 (21.4%) out of 1450 examined fecal sample were positive for *Fasciola spp.* eggs and also, the postmortem examination of

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1450 sheep found that 386 (26.6%) were harboring *F.gigantica,* whereas the results obtained by **Haridy *et al,* (1999)** through investigation overall sheep and goats slaughtered in Egyptian abattoirs between 1994 and 1997 for presence of fascioliosis was indicated that 40.465 (2.02%) out of 2.003.200 examined animals, also the result was lower than result obtained by **Khalafallah, (2000)** who examined sheep postmortem and found that 5 (2.9%) out of 173 had *F.gigantica* infection, also greatly lower than result obtained by **Awadallah, (2001)** who used P.M. examination his results revealed that 24 (17.52%) out of 137 examined sheep was harbouring *F.gigantica* infection, while result obtained by **Ibrahim *et al,* (2002)** through examination of 300 liver of slaughtered sheep revealed that 25 (8.4%) had *F.gigantica* infection, this may be attributed to sheep owners now days stick to routine treatment with one or more of fascioloicidal drugs and due to significant decrease in the population of snail intermediate host for *Fasciola spp.* in the last few years.

The prevalence rate of adult cestodes was 39 (20.64%) out of 189 sheep, so that it slightly lower than results obtained by **Keedes, (1990)** whose results indicated that 8 (23.5%) out of 34 sheep had an infection with cestode parasite, also it was lower than results obtained by **Abd El-Miguid, (1998)** who stated that 79 (39.5%) out of 200 examined intestinal tract of sheep had cestode worms, while it was markedly lowered than that of **Khalafallah, (2000)** whose results indicated that 48 (27.7%) out of 178 sheep had an infection with cestode, and this may be due to changing of feeding behavior of sheep and routine treatment of sheep.

The prevalence rate of *Moneizia expensa* was 36 (19.04%) out of 189 sheep, this result agreed with that of **Khalafallah, (2000)** who stated that 34 (19.7%) out of 173 examined sheep had infection with *M.expensa,* but this result was higher than this obtained by **El-Sayed, (1991)** who stated that 54 (14%) out of 386 sheep had *Moneizia spp.* infection.

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The prevalence rate of *Cysticercus teniucollis* was 32 (16.93%) out of 189 sheep, this result was lower than result of **Pathak and Gaur, (1982)** who stated that 300 (37.03%) out of 810 sheep, **El-Massry, (1999)** who examined 500 sheep sera by using ELISA and found 151 (30.2%) were seropositive, although by P.M. examination only 100 (20%) animals were harbouring *C.teniucollis,* lower than results of **Kandil *et al*, (2005)** who found that 33 (27.5%) out of 120 sheep were harbouring *C.teniucollis*, but agreed with that result obtained by **Hasslinger and Weber-Werringhen, (1988)** as they found that 785 (16.7%) out of 4710 slaughtered sheep had *C.teniucollis* also agreed with results of **Abdel-Maogood, (2005)** who found that 45 (16.98%) out of 200 examined slaughtered sheep had *C.teniucollis.*

The prevalence of nematode helminthes infecting sheep was 22 (11.64%) out of 189 sheep, this result was lower than those results obtained by **Hassona, (1979)** who depends mainly on P.M. examination of abomasa of 350 sheep, out of examined sheep 124 (35.248%) were harbouring abomasal worms, **Hassan, (1988)** who depends on coprological examination in his work and found that 1157 (55.13%) out of 2100 sheep were infected with nematode eggs and the most common type of egg was of members of family *Trichostrongylidae*, **El-Fauomy, (1989)** who found that 60 (10%) and 381 (63.5%) out of 600 fecal sample of sheep were positive for eggs of *Strongyloides* and *other strongyles* respectively, **Kedees, (1990)** who found that 10 (29.4%) out of 34 sheep intestinal tract had infection with nematode, **El-Fauomy, (1992)**whose results indicated that 117 (45.7%) out of 256 examined abomasa of slaughtered sheep were harbouring either single or mixed infection with abomasal nematodes, and **Abd El-Miguid, (1998)** his results indicated that 112 (56%) out of 200 examined sheep had an infection with nematodes, but the obtained result in this study was higher than the result obtained by **Khalafallah, (2000)** who found that 40 (23.1%) out of 173 examined sheep in abattoir.

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Concerning the prevalence rate of *Haemonchus contortus* was lower than those of **Hassona, (1979)** who result indicated that 136 (38.7%) out of 350 abomasa of sheep had infection with *H.contortus* and results obtained by **El-Sayed, (1992)** who 44 (17.18%) out of 256 abomasa of sheep had an infection with *H.contortus*, but the obtained result was higher than results obtained by **Khalafallah, (2000)** 6 (3.5%) out of 173 examined sheep by P.M. examination were infected with *H.contortus.*

Results of prevalence of mixed infection in examined sheep indicated that 24 (12.7%) out of 189 had mixed infection by more than one species of helminthes, this result was markedly lower than this obtained by **Abd El-Miguid, (1998)** who examined 200 intestinal tract of sheep, among these 64 (44.44%) were infected with more than one helminth parasites, and also was lower than result of **Khalafallah, (2000)** who found that 19 (11%) out of 173 examined sheep had a mixed infection with more than one helminth parasites.

While ELISA results by using *H.contortus* crude somatic antigen (CSA) showed that, its sensitivity was 87.5%, this may be attributed to the type of antigen which used was crude non-purified, non-characterized antigen, this results agreed with **Handrilix, (1990)**, **Schallig, (1994)** who found that by using *H.contortus* CSA in ELISA assay the sensitivity was 89.2% , but disagree with result of **Derbala** and **Abdel-Rahman, (2001)** as they stated that coproantigen is more more potent in diagnosis of sheep haemonchosis than somatic and circulating antigens of *H.contortus.*

While ELISA result by using *C.teniucollis* whole cyst antigen showed that the sensitivity was 90% , this result agreed with **El-Massry ,(1999)** her results showed that out of 100 case were harbouring *C.teniucollis* in P.M. examination, only 91 were seropositive with ELISA assay.

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Although, ELISA assay is new, rapid, easy, sensitive and specific assay to be used in diagnosis of infections especially parasitic infections, but its results depends mainly on the type of antigen which used and sera which used as a control positive and/or negative, in few words to obtain the best results, should use specific, purified antigen with positive control hyperimmune sera prepared in suitable lab animal and the negative control sera preferred to be sera of another host rather than animal species in the research.

Histopathological studies indicated that the liver of the naturally infected sheep showed fibrosis, many accessory ducts formed and leukocytic infiltration by lymphocytes, these results agreed with those obtained by **Ibrahim, (2002)** and **El-Dakhly, (2003).**

Heavy leukocytic infiltration was observed and most of the infiltrated cells were esinophils and macrophages, these results agreed with those obtained by **El-Dakhly, (2003)**.

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VI- Summary

189 sheep of different breeds, sex and ages were examined during post-mortem examination for presence of different helminthes, results showed that 98 (51.9%) sheep were infected with helminthes, sheep harbouring trematodal infection were only 5 (2.65%), while those harbouring adult cestodes were 39 (20.64%), sheep infected with *C.teniucollis* were 32 (16.93%) and sheep harbouring abomasal nematodes were 22 (11.64%).

Fasciola gigantica was recovered from only 1 sheep (0.53%), *Paramphistomum cervi* was recovered from 4 sheep (2.12%) , *Moneizia expensa* was recovered from 36 sheep (19.04%) and *Avitellina centripunctata* was recovered from 3 sheep (1.6%), moreover *Haemonchus contortus* was the most predominant recovered nematode species from 15 (7.9%) sheep, other nematodes species i.e. *Parabonema skrjabini* and *Graphidiops spp.* recovered from 7 (3.7%) sheep.

The incidence rate of mixed infection was 12.698% (24 out of 189), the most common type of mixed infection was mixed infection with *Haemonchus contortus* and other species of abomasal nematodes i.e. *Parabonema skrjabini* and *Graphidiops spp.* its incidence rate was 41.7% (10 cases out of 24 cases of mixed infection), while mixed infection with *Moneizia expensa* and *C.teniucollis* incidence rate was 29.16% (7 cases out of 24 cases of mixed infection), mixed infection with *Avitellina centripunctata* and *C.teniucollis* incidence rate was 8.33% (2 cases out of 24 cases of mixed infection).

After preparation of different antigens from different collected helminthes, the protein content of each was determined.

By using crude *Haemonchus contortus* antigen in ELISA (at concentration 40 µg/ ml coating buffer) indicated that the sensitivity was 87.5%, and by using *Cysticercus tenuicollis* whole cyst antigen at concentration (40 µg/ ml coating buffer) revealed sensitivity was 90%.

Histopathological examination of liver infected with *Fasciola spp.* indicated that presence of sever fibrosis and periductal hyperplasia, heavy leukocytic infiltration, the most infiltrated cells were esinophils and macrophages.

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VII- Conclusion

Examination of 198 slaughtered sheep for presence of different helminthe parasites, showed that 98 (51.9%) sheep were infected with different helminthes.

Adult cestode infection was the most common helminthe infection among examined sheep as adult cestode parasites recovered from 39 sheep (20.64%), followed by infection with larval stages of *T.hydatigena (C.teniucollis)* which recovered from 32 sheep (16.93%), while infection abomasal nematodes was counted in 22 sheep(11.64%), infection with trematode parasites was the lowest infection and were only in 5 (2.65%) sheep.

Regarding the identified species, *Moneizia expensa* was the most recovered species as 36 sheep (19.04%) were harbouring *M.expensa* in their intestinal tract, followed by *C.teniucollis)* which recovered from 32 sheep (16.93%), moreover *Haemonchus contortus* was the most predominant recovered nematode species as it was collected from 15 (7.9%) sheep abomasa, other thread worms i.e. *Parabonema skrjabini* and *Graphidiops spp.* were collected from 7 (3.7%) sheep abomasa, while trematodes worms which recovered were *Paramphistomum cervi* which recovered from 4 sheep (2.12%) and *Fasciola gigantica* which recovered from only 1 sheep (0.53%),

The incidence rate of mixed infection was 12.698% (24 out of 189), the most common type of mixed infection was mixed infection with *Haemonchus contortus* and other species of abomasal nematodes i.e. *Parabonema skrjabini* and *Graphidiops spp.* its incidence rate was 41.7% (10 cases out of 24 cases of mixed infection), while mixed infection with *Moneizia expensa* and *C.teniucollis* incidence rate was 29.16% (7 cases out of 24 cases of mixed infection), mixed infection with *A vitellina centripunctata* and *C.teniucollis* incidence rate was 8.33% (2 cases out of 24 cases of mixed infection).

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By using crude *Haemonchus contortus* antigen in ELISA (at concentration 40 µg/ ml coating buffer) indicated that the sensitivity was 87.5%, and by using *Cysticercus tenuicollis* whole cyst antigen at concentration (40 µg/ ml coating buffer) revealed sensitivity was 90%.

Histopathological examination of liver infected with *Fasciola spp.* indicated that presence of sever fibrosis and periductal hyperplasia, heavy leukocytic infiltration, the most infiltrated cells were esinophils and macrophages.

In a few words, until nowadays helminthe parasites infecting sheep is still a hazard that should be in mind of owners, veterinarians and researchers as, although, serious infections such as infection with *Fasciola spp.* and infection with *Haemonchus spp* limited, but other less serious infections such as infection with *Moneizia spp.* and infection with *C.teniucollis* are emerging.

Recent immunological techniques e.g. ELISA assay are the hope and the way to make a progress in both immunodiagnosis and/or immunoprophylaxis of such infections

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العلاقات البينية المناعية بين بعض الديدان في الأغنام

الملخـــص العربـــى

من المعلوم أن الأغنام تعتبر من الحيوانات الواعدة لتحقيق الاكتفاء من المنتجات ذات الأصل الحيوانـي من لحوم حمراء وألبان وصوف ولقد زادت قطعان الأغنام عدداً وزاد إنتاجيتها في الفـترة الأخـيرة مقارنـة بزيادة عدد السكان وخصوصاً في مصر ، وتشكل الأمراض الطفيلية وخصوصاً العدوى بالديدان عـامل مـهم ومؤثر في إنتاجية الأغنام المعرضة للإصابة بحوالي ٢٠ نوع مختلف من الديدان.

ولقد أجريت دراسات عديدة علي ديدان الأغنام والهدف من هذه الدراسة استبيان وجود علاقات بينيـة مناعية بين بعض أنواع الديدان التي تصيب الأغنام مثل (الفاشيولا جيجانتيكا و المونيزيـا و السيستسـركس تينيكولاس و الهيمونكس).

ولهذا فقد أجريت هذه الدراسة على فحص عدد ١٨٩ ذبيحة من الأغنام مختلفة السـلالات والأعمـار والأجناس ، وقد تم الكشف علي هذه الذبائح للبحث عن الأنواع المختلفة من الديدان والتـي يتـم تجميعـها وتجميع عينات دم بدون مانع تجلط بغرض فصل السيرم نقل هذه العينات إلى المختبر بكلية الطـب البيطـرى بكفر الشيخ للتعامل معها بالطرق العلمية المعروفة.

و أظهرت النتائج أن نسبة الإصابة بالأنواع المختلفة من الديدان فى الأغنام المفحوصة تصل إلى نسبة ٥١,٩% (٩٨ من ١٨٩) منها ٢,٦٥% (٥ من ١٨٩) ديدان مثقوبة (تريماتودا) ،٢٠,٦٤% (٣٩ من ١٨٩) ديدان شريطية بالغة، ١٦,٩٣% (٣٢ من ١٨٩) يرقات لديدان شريطية (يرقات ديدان التينيـا هيداتيجنيـا والمعروفة تحت الاسم العلمي السيستسركس تينيكولاس) ، ١١,٦٤% (٢٢ من ١٨٩) ديدان اسطوانية معديـة (خيطية).

وقد قام الباحث بتصنيف أنواع الديدان المجمعة طبقاً للأساليب والمراجع العلمية المعروفـة وسـجلت نسبة الإصابة لكل نوع منها ، فكانت نسبة ديدان الفاشيولا جيجانتيكا (الـدودة الكبديـة العملاقـة) ٠,٥٣% (١ من ١٨٩) ، ديدان الكرش من نوع البارمفيستومم ٢,٢١% (٤ من ١٨٩) ، و كانت نسبة الإصابة بديدان المونيزيا١٩,٠٤% (٣٦ من ١٨٩) ، وبالنسبة لنوع الافتيلاينا ١,٦% (٣ من ١٨٩) ، أما نوع الـهيمونكس كونتورتس فكانت نسبة الإصابة به ٧,٩% (١٥ من ١٨٩) ، أما بالنسبة للأنـــواع الأخـرى مـن الديـدان

الاسطوانية المعدية فكانت الأنواع المكتشفة هما نوعا الجرافيدوبيس ، الباروبرنيما إسكربيني وكانت نسبة الإصابة بهما ٣,٧% (٧ من ١٨٩).

و كانت نسبة الإصابة المختلطة بأكثر من نوع واحد من الديدان فكانت ٢٤,٥% (٢٤ من ٩٨) ، وكانت أعلى نسبة إصابة من العدوى بأكثر من نوع هى العدوى بالهيمونكس مع النوعين الآخرين من الديدان الاسطوانية المعدية بنسبة ٤١,٠٧% (١٠ من ٢٤) ، وسجلت نسبة الإصابة المختلطة بديدان المونيزيا والسيستسيركس تينكولاس نسبة ٢٩,١٦% (٧ من ٢٤) ، أما نسبة الإصابة المختلطة بديدان الافتيلينا والسيستسيركس تينكولاس فكانت ٨,٣٣% (٢ من ٢٤) ، أما بالنسبة للعدوى المختلطة الاخرى مثل العدوى بالفاشيولا مع الهيمونكس او الهيمونكس مع كل من المونيزيا او الافتيلينا و اخيرا السيستسيركس تينيكولاس مع الهيمونكس فسجلت كل منها فى حالة واحدة فقط بنسبة ٤,١٦% (١ من ٢٤) لكل منها.

ثم قام الباحث بتحضير الانتجينات لكل من الديدان المجمعة بالطرق العلمية المناسبة لكل نوع وتم قياس نسبة البروتين الكلى الموجود فى كل انتجين منها باستخدام الطريقة العلمية المناسبة ، وقد تم اختيار أكثرها مناسبة من حيث كمية البروتين الكلى الموجود.

وباستخدام اختبار الإليزا وجد أن باستخدام الانتجين الخام لدودة الهيمونكس أعطت حساسية ٨٧,٥% أما الانتجين الكلي للسيستسركس تينيكولاس فقد أعطى حساسية ٩٠% .

أما نتائج الفحص (الفحص التشريحى) الميكروسكوبى للأنسجة (الهستوباثولوجى) للكبد المصاب بديدان الفاشيولا جيجانتيكا فقد أظهرت زيادة فى التليف مع زيادة فى عدد كريات الدم البيضاء و خصوصاً الأكلة منها.

جامعة طنطا –فرع كفر الشيخ

كلية الطب البيطرى

قسم الطفيليات

قرار لجنة الحكم والمناقشة

قـررت لجـنة الحكم والمناقشة بجلستها المنعقدة يوم الثلاثاء الموافق ٢٠٠٦/٥/٩ ترشيح السيد ط.ب./ خالد مسعد إبراهيم سلطان للحصول على درجة الماجستير فى العلوم الطبية البيطرية تخصص الطفيليات تحت عنوان" العلاقات البينية المناعية بين بعض الديدان فى الأغنام ".

*أعضـــــاء اللجنـــــة:-

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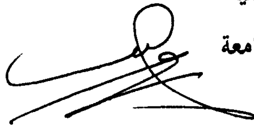

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"العلاقات البينية المناعية بين بعض الديدان فى الأغنام"

رسالة مقدمة من

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بكالوريوس العلوم الطبية البيطرية - كلية الطب البيطرى - كفر الشيخ -٢٠٠٢م.

للحصول على

درجة الماجستير فى العلوم الطبية البيطرية

تخصص (طفيليات)

تحت إشراف

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جامعة طنطا

للحصول على

درجة الماجستير في العلوم الطبية البيطرية

تخصص طفيليات

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