Application of Monoclonal Antibodies in Veterinary Parasitology

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Abstract

The discovery of hybridoma technology by Kohler and Milstein in 1975, heralded a new era in antibody research. Mouse hybridomas were the first reliable source of monoclonal antibodies. The generation of monoclonal antibodies from species other than rats and mice, has developed slowly over the last 30 years. The advent of antibody engineering and realization of the advantages of non murine antibodies has increased their relevance recently. However, in the area of veterinary parasitology, monoclonal antibodies are just beginning to fulfill the promises inherent in their great specificity for recognizing and selectively binding to antigens. This review describes the recent advances in the application of monoclonal antibodies for immunodiagnosis / prophylaxis and immunotherapy of parasitic diseases.

Keywords: - Coccidiosis, Fasciolosis, Immunodiagnosis, Immunotherapy, Monoclonal antibody, Vaccine, Parasite, Parasitology, Hybridoma.

Introduction

The advent of biotechnology has invigorated research on the control of parasitic diseases. A thorough study of parasite antigens is a prerequisite for control programmes based on protection by vaccination, accurate serodiagnosis and perhaps immune modulation to diminish pathological sequelae. The utilization of hybridoma technology to produce antigen-specific monoclonal antibodies (Mabs) has resulted in great strides towards obtaining pure antigens relevant for immunodiagnostic purposes and for research on vaccines. This technology was discovered in 1975 by two scientists Georges Kohler of West Germany and Caesar Milstein of Argentina who jointly with Neil Jerne of Denmark were awarded the noble prize for Physiology and Medicine.

Advantages of Monoclonal Antibodies

Each Mab recognizes only one antigenic determinant or epitope. Therefore, a Mab may be selected which has specificity for an antigenic determinant unique to a particular parasite and false positive reactions due to cross-reactivity are minimized or eliminated.

While diagnostic reagents vary from laboratory to laboratory but the parasite antigen recognized or isolated using a Mab is invariant, thus permitting reproducibility and standardization in diagnostic tests. All the antibodies produced (100%) are active antibodies therefore high specific activity of labelling is possible in radioimmunoassay, enzyme linked immunosorbent assay and fluorescent immunoassay. Mabs could be advantageously used as immunosorbent for antigen purification using imunosorbent column chromatography.

Large amount of Mabs (1-20 mg per ml ascites fluid) could be obtained with modest investment. Mabs specific for a particular target antigen can be obtained even without prior purification of antigen.

Applications in Protozoal Infections

Coccidiosis: Coccidiosis is the most important parasitic disease of poultry resulting in over $ 300 million in annual losses. Recent studies on Eimeria using monoclonal antibodies have laid the ground work for isolating functional antigens in immunization trials in chickens. The characterization and localization of antigens of Eimeria spp. by monoclonal antibodies (MAb) has focused predominantly on surface antigens of sporozoites and merozoites or specific organelles such as sporozoite refractile bodies. The monoclonal antibodies produced against Eimeria tenella recognized all strains of this species that were tested but did not react with any of six other Eimeria species.

To assess the role of specific antigen of *E. tenella* in infectivity, the effect of three distinct monoclonal antibodies on parasitic establishment and development

was studied in vitro (Danforth, 1983). All three antibodies influenced the ability of parasites to enter cells by 24 hrs and nearly completely inhibited the invitro development of the parasites by 96 hrs. These studies suggest that all the three antigens recognized by the different monoclonal antibodies are important for the intracellular establishment and development of *E. tenella*. Although the precise nature of establishment and development has not been determined, other studies indicate that one of the monoclonal antibodies, recognizing a 22K sporozoite antigen, binds to the sporozoite surface and in the presence of complement, mediate cell lysis (Danforth et al.,1985; Danforth, 1986) supporting the immunotherapeutic use of hybridoma technology in poultry coccidiosis.

Since the sporozoite antigens identified by hybridoma antibodies, were found in such minute amounts that it become necessary to utilize genetic engineering in order to produce enough protein for immunization and one such protein, designated 5401 has been shown the use of genetically engineered antigen as potential vaccine candidates against coccidiosis. The antibodies production in birds has been elicited and partial protection against coccidial infection has also been produced. The progress obtained from these investigations makes the development of vaccine against coccidia by use of birds own protective immune response within the realm of possibility (Danforth and Augustine, 1985). Some work has also been done on the stage cross reactive Mab by use of indirect immunofluroscent antibodies (IFA) and western blot analysis. One such cross reactive monoclonal antibody designated 1205 was used to study redistribution, parasitophorus vacuole incorporation and *insitu* antigen production during the intracellular parasite development of *Eimeria acervulina* and *E. tenella* (Danforth et al., 1994).

Babesiosis: Babesia (causing babesiosis or Red water fever) is an intraerythrocytic protozoan parasite of livestock transmitted by a tick intermediate host. Monoclonal antibodies generated against the crude parasite fractions of Babesia bovis have been used to identify and isolate pure parasite antigens. One of these antigens, of 44K molecular weight, has been used to protect cattle against virulent challenge (Wright et al., 1983, 1985). These results suggest that a candidate antigen has been identified with potential as a defined vaccine for bovine babesiosis.

Monoclonal antibodies designated as BC5.37. 70.27 (BC5) and BE1.24/2.95 (BE1) that recognized the 70 kDa and 34 kDa protein of Babesia caballi and Babesia equi respectively were successfully produced (Bruning et al., 1997). Both Mabs reacted specifically in indirect ELISA when isolated whole merozoites were used as antigens. Similarly, MAb BEG3 produced against a species specific 19 kDa antigen of *B. equi* significantly inhibited the invitro growth of *B. equi* parasite (Avarzed et al., 1998).

Monoclonal antibodies directed against a 58 kDa Babesia bigemina merozoite antigen reacted strongly with immune sera from experimentally and naturally infected cattle in Western Blots (Molly et al., 1998). Further, competitive inhibition ELISA detected antibodies directed against a single epitope on the 58 kDa antigen with 95.7% sensitivity and 97% specificity. In calves experimentally infected with B. bigemina, the seroconversion was detected at about 10 days post inoculation. The test can be useful for the epidemiology studies, particularly in areas where *B. bovis* and *B. bigemina* have overlapping distributions.

Theileriosis: Theileria parva is an intracellular protozoan parasite that causes a disease termed East Coast fever in cattle in East and Central Africa. Cattle become infected when sporozoites of *T. parva* are secreted into the blood during feeding of the tick intermediate host.

Monoclonal antibodies have been developed that identify common antigenic determinants on the surface of several strains of *T. parva* sporozoites. These antibodies have been found to neutralize the infectivity of sporozoites in an invitro assay (Dobberlaere et al., 1984; Musoke et al., 1984) and invivo when sporozoites are pretreated with Mabs prior to inoculation into cattle. One of these protective monoclonal antibodies was subsequently used to isolate a 68K antigen from the surface of *T. parva* sporozoites (Dobbelaere et al., 1985). These results indicate that sporozoite surface antigens common to different stocks of *T. parva* may have potential as a broad spectrum vaccine; however, field testing of these antigens will undoubtedly require antigen production by gene cloning on other methods.

Toxoplasmosis: *Toxoplasma gondii* is an intracellular protozoan parasite that infects virtually all warm-blooded animals and is of considerable public health importance. The antigens of *T. gondii* have been the subject of considerable research aimed at improved diagnosis and vaccine development. Monoclonal antibodies have been used to identify antigens from the surface (Handman et al., 1980; Sethi et al., 1980; Jhonson et al., 1981; Kaspar et al., 1983) and the cytoplasm (Sharma et al., 1984) of the intracellular tachyzoite stage. Antibodies directed

against a 30K molecular weight tachyzoite surface antigen have been used to kill parasites invitro in the presence of complement (Kaspar et al., 1983). However, active immunization with the purified antigen failed to confer any protection (Kaspar et al., 1985). Another study showed that monoclonal antibodies directed against 35K and 14K molecular weight tachyzoite surface antigens were able to confer passive protection in mice (Jhonson et al., 1983). A monoclonal antibody directed against a 58K molecular weight tachyzoite cytoplasmic antigen was found to confer passive protection and the affinity-isolated antigen conferred complete protection against lethal challenge in mice (Sharma et al., 1984). Murine Mabs against Neospora caninum tachyzoites were produced to identify the cross-reactive antigens between *N. caninum* and *T. gondii* and it is reported that some of the proteins could be useful in developing vaccines or drugs for controlling the diseases caused by the two parasites (Liao et al., 2005).

Cryptosporidiosis: *Cryptosporidium parvum* is an obligate enteric protozoan parasite which infects the gastrointestinal tract of animals and humans. It is transmitted by the fecal–oral route via the oocyst stage through ingestion of contaminated water or food, direct contact with infected humans or animals, or contaminated surfaces. A number of zoite surface glycoproteins are known to be expressed and believe to be involved in invasion and infection of host epithelial cells. In the absence of protective treatment for this illness, antibodies targeted against these zoite surface glycoproteins offers a rational approach to therapy. Mabs against sporozoite surface antigens have proven useful in the characterization of unique antigens and in detection of oocysts from feces for diagnostic purposes (Anusz et al., 1990). The major goals for developing anti-C. parvum Mab were - first is Mabs against sporozoite antigens can inhibit parasite invasion and lessen the severity of infection in animals (Tilley et al., 1991) and secondly, Mabs that target oocyst-wall antigens have been valuable in detection of Cryptosporidium in fecal material and for determination of the extent of oocyst contamination in water supplies (Smith and Rose, 1990; Sterling and Arrowood, 1986).

Despite the advantages of Mabs, targeting single antigenic determinants may be inadequate when dealing with multiple antigenic determinants involved in host - *C. parvum* interaction and result in ineffective therapy. This disadvantage could be overcome by using a combination of Mabs each targeting different antigenic sites or by using polyclonal antibodies. In addition, Cryptosporidium infectivity in experimentally infected animals was decreased with the use of immune colostrum in combination with Mabs (Perryman et al., 1990).

Helminth Infection

Fasciolosis: Very few studies have been reported on the production of monoclonal antibodies to Fasciola spp. with most work on Fasciola hepatica rather than *Fasciola gigantica*. These include monoclonals which were reactive with tegumental antigens present in the tegument-I (TI) granules and glycocalyx of the fluke (Hanna and Trudgett, 1983; Hanna et al., 1988) and monoclonals against some excretory-secretory (E/S) products of F. hepatica (Solano et al., 1991).

A monoclonal antibody-based capture ELISA was developed for detection of a 26 to 28 kDa antigen of F. hepatica in the feces of infected cattle (Abdel et al., 1998). Mab was recognized as M2D5/D5F10. This test is highly specific and sensitive and it can diagnose the infection as early as 6 weeks duration and can detect as little as 300 pg of coproantigens/ml of fecal supernatant. The assay results correlated well with the number of flukes suggesting that it is possible to estimate fluke burden.

MM3 Mabs, produced by immunizing mice with a 7 to 40 kDa purified and O-deglycosylated fraction of F. hepatica E/S antigen, were used for the detection of F. hepatica E/S antigens in feces of infected hosts (Mezo et al., 2004). This experiment indicated that capture ELISA using Mab MM3 assay is a reliable and ultra sensitive method for detecting subnanogram amounts of F. hepatica antigens in feces from sheep and cattle, facilitating early diagnosis. The MM3 capture ELISA assay detected 100% of sheep with one fluke, 100% of cattle with two flukes and 2 of 7 cattle with one fluke. The false negative animals (5/7) were probably not detected because the *F. hepatica* in these animals was immature.

MM3 sero and MM3 copro ELISA test in sheep experimentally infected with *F. hepatica* or *F. gigantica* showed that fasciolosis could be detected at 4 weeks post infection from serum samples and copro antigens could be detected at 4-7 weeks in *F. hepatica* and 3-6 weeks in *F. gigantica* (Valero et al., 2008). The study demonstrated that MM3 sero and MM3 copro ELISA could be applied in the studies on epidemiology, pathogenesis and treatment in animals and humans where *F. hepatica* and *F. gigantica* co-exists.

A 28 kDa *F. gigantica* cysteine proteinase (FgCL-3) was purified from the E/S product of

bubalian origin flukes with an average yield of 116.75µg/ fluke (Dixit et al., 2003). Fagbemi and Hillyer (1992) previously isolated a 28 kDa protease from whole worm extract of F. gigantica. It is not clear that the 28 kDa recognized in whole worm extract is the same as that in the E/S products. Fagbemi (1995) also produced Mabs which were reactive with the 28 kDa protease and a 27/28 kDa doublet of *F. gigantica.* The 28 kDa protease specific Mab could be a useful tool for direct isolation of the protease from the whole worm extract and/or E/S products of *F. gigantica* by antibody affinity chromatography. These proteases possess immuno-diagnostic properties and showed 100% sensitivity in detection of low and high grade experimental fasciolosis in sheep and buffaloes (Dixit et al., 2002, 2004). However, under field situation of natural F. gigantica infection, this sensitivity of the test declined to 97% but specificity remained 100% (Raina et al., 2006).

Most of E/S proteases which are 26-30 kDa in size resemble mammalian liver Cathepsin-L proteases both in amino acid sequence and substrate specificity (Brady et al., 1999; Tort et al., 1999). Currently, these are referred as the *Fasciola Cathepsin*-L cysteine proteinases (Dixit et al., 2008). First 14-N terminal aminoacids of 28 kDa *F. gigantica* cathepsin L cysteine protease (FgCL-3) on sequence analysis showed an identity of 64% with N- terminal sequence of recombinant FgCL-1(rFgCL-1) produced by Grams et al. (2001). The Mab 2B11-E8-E3 was developed against recombinant F. gigantica Cathepsin L-A (rFgCL-A). This Mab detected 0.8ng of rFgCL-A and specifically reacted with 30 kDa rFgCL-A and native 28-29 kDa Cathepsin –L in E/S products and crude worm extract of *F. gigantica.* Immunolocalization by Mab found Cathepsin –L in the epithelial lining of gut (Inthakanok, 2007).

Cystic Echinococcosis: Cystic echinococcosis is an endemic cosmopolitan zoonotic helminthic disease caused by the larval stage of *Echinococcus granulosus*. Two hybridomas were produced using spleen cells from mice experimentally infected with hydatid parasite *E. granulosus* (Craig et al., 1981). These two hybridomas secreted antibody with anti-*E. granulosus* cyst fluid (EgCF) activity. However, these monoclonal antibodies produced against *E. granulosus* cross react with sera from Taenia ovis, *Taenia hydatigena* and *F. hepatica* infected sheep. Anyhow the partially purified *E. granulosus* antigen showed improved specificity in an indirect ELISA for the diagnosis of infected sheep.

Khaled et al. (2008) considered antigen B (AgB), a thermostable lipoprotein that constitutes a considerable fraction of the cystic hydatid fluid, as a suitable source for vaccination and immunodiagnosis of cystic echinococcosis due to its high sepecificity. They further reported that the genetic immunization of BALB/c mice with second subunit of antigen B (Eg AgB8/2) for the production of monoclonal antibodies. Fusion products between spleen cells and myeloma cells produced six MAbs. All MAbs identified the four AgB subunits with molecular weights of 8, 16, 24 and 36kDa. Further work on specificity and sensitivity of these MAbs as well as their use in detecting circulating parasite antigens and in antigen purification will be assessed in future studies.

Dirofilariosis: Monoclonal antibodies were prepared for the detection of parasite antigens in the serum of *Dirofilaria immitis* infected dogs by counter immuno electrophoresis (Weil, et al., 1985). Monoclonal antibodies were also generated against adult worms for the identification of circulating antigens associated with immune-complex glomerulo-nephritis in dogs infected with *D. immitis*. Using these antibodies antigen-capture ELISA detected circulating antigens in 75% of infected dogs. Two bands of 62 and 28kDa respectively were detected on western blot by monoclonal antibody. Parasite antigen detection with the Mab-based ELISA appears to be superior to previously described diagnostic methods for canine dirofilariosis in terms of sensitivity, specificity and in relation to infection intensity (Nakagaki et al., 1993).

Trichinosis: Studies in rodents have shown that antigens of immunological value may be derived from three stages of the life cycle of *T. spiralis*, the tissue-dwelling muscle larvae, the intestinal adult stage and the migrating newborn larvae (Wakelin and Denham, 1983). Monoclonal antibodies against the muscle larvae stage of *T. spiralis* were produced by fusing spleen cells from mice inoculated orally with infective parasites (Gamble and Graham, 1984). Most monoclonal antibodies recognized antigens shared by larval and adult stage of *T. spiralis* and by other swine parasites, particularly *Trichuris suis*. However, several monoclonal antibodies were specific for *T. spiralis* larvae or adult antigens. Because of the demonstrated diagnostic value of assays utilizing larval ES products, one hybridoma with specificity for an ES antigen of muscle larvae (7C2C5) was selected for further study. The Mab 7C2C5 recognized a single antigen epitope present on proteins of molecular wt. 45000, 49000 and 53000 (Ts45, Ts49, Ts53). These antigens were isolated by affinity-chromatography and used in a triple-antibody indirect ELISA for the

detection of antibodies to *T. spiralis* (Gamble et al., 1983).

Because of time and expense involved in affinity isolation of antigen, Gamble and Graham (1983) developed a competitive immuno assay using 7C2C5. No false positive reactions were obtained and no cross reactions were obtained with serum pools from hogs infected with *Ascaris suum*, *Strongyloides ransomi* or *Stephanurus dentatus* or from hogs with serum antibody titres against *Trichuris suis*. Therefore, this assay appears to offer a more simple but still sensitive and specific test for swine trichinosis.

Monoclonal antibodies that recognized surface antigens of approximately 64 K molecular weight marked (NIM-M5; IgGl) in newborn larvae (NBL), mediated the adherence of rodent eosinophil leucocytes to the surface of living NBL. Following cell adherence, the worms were killed (Ortega-Pierres et al., 2007). Thus a single monoclonal antibody to NBL was able to mediate eosinophil-dependent destruction of worms invitro and reduce infectivity invivo. These observations suggest that antibodies capable of mediating eosinophil-induced destruction of nematodes invitro may also be important in protection against infection.

Conclusion

As failure to detect low level patent infection incurs the risk of having a reservoir capable of perpetuating infections, there is an urgent requirement for acute serodiagnosis for parasite control programmes. Hybridoma technology is just beginning to be applied in the field of parasites immuno-diagnostic and the recent success in research employing these techniques should encourage greater interests. Preliminary reports indicate that the monoclonal antibodies with immunodiagnostic value have been produced against many important parasites. The use of monoclonal derived reagents have greatly increased the specificity of diagnosis by eliminating cross reactions between closely related parasite spp., without suffering a significant loss of sensitivity. It is hoped that continuing research along with the use of new antibody- based strategies for immunodetection, prophylaxis and immunotherapy will pave new means to combat the notorious parasites.

References

1. Abdel-Rahman, S.M., Reilly, K.L.O. and Malone, J.B. (1998), Evaluation of a diagnostic monoclonal antibody-based capture enzyme-linked immunosorbent assay for detection of 26- to 28-kda Fasciola hepatica coproantigen in cattle. *Am. J. Vet. Res*., 59:533-7.
2. Anusz, K.Z., et. al. (1990), Detection of *Cryptosporidium parvum* oocysts in bovine feces by monoclonal antibody capture enzyme-linked immunosorbent assay. *J. Clin. Microbiol*., 28: 2770–4.
3. Avarzed, A., Igarashi , I., De Waal, D.T., Kawai, S., Oomori, Y., Inoue, N., Maki, Y., Omata, Y., Saito, A., Nagasawa, H., Toyoda, Y. and Suzuki, N. (1998), Monoclonal antibody against Babesia equi: characterization and potential application of antigen for serodiagnosis. *J. Clin. Microbiol.*, 36 (7):1835-9.
4. Bruning, A., Phipps, P., Posnett, E. and Canning, E.U. (1997), Monoclonal antibodies against Babesia caballi and Babesia equi and their application in serodiagnosis. *Vet. Parasitol.*, 68(1-2):11-26.
5. Brady, M.T., O'Neill, S.M., Dalton, J.P. and Mills, K.H.G. (1999), Fasciola hepatica a protective Th1 response against Bordetella pertussis. *Infect. Immun.*, 67: 5372-5378.
6. Craig, P.S., Hocking, R.E., Mitchell, G.F. and Rickard, M.D. (1981), Murine hybridoma-derived antibodies in the processing of antigens for the immunodiagnosis of hydatid (Echinococcus granulosus) infection in sheep. *Parasitology*, 83(2):303-17.
7. Danforth, H. D. and Augustine, P.C. (1985), Use of hybridoma antibodies and recombinant DNA technology in protozoa vaccine development. *Avian Dis.*, 30 (1): 37-41.
8. Danforth, H. D., MacAndrew, S. J. and Augustine, P. C. (1985), Characterization of avian coccidial antigens recognized by hybridoma antibodies. *Proc. Fed. Am. Soc. Exp. Biol.*, 44: 1334.
9. Danforth, H.D. (1983), Use of antibodies directed against Eimeria tenella sporozoite to determine stage specificity and in vitro effect on parasite penetration and development. *Am. J. Vet. Res.*, 44: 1722-1727.
10. Danforth, H.D. (1986), Use of hybridoma antibodies combined with genetic engineering in the study of protozoan parasites: A review. In: L. P. Joiner, P. L. Long and L. R. McDougald (Ed.) Proc. Georgia Coccidiosis Conf., p 574.
11. Danforth, H.D., Augustine, P.C., Barta, J.R. and Jenkins, M.C. (1994), In vitro and In vivo immunolabeling of sporozoites, schizonts and sexual stages of Eimeria acervulina and E. tenella by a species and stages cross-reactive monoclonal antibody. *Parasitol. Res.*, 80: 594-599.
12. Dobbelaere, D.A.E., Spooner, P. R., Barry, W. C. and lrvin, A. D. (1984), Monoclonal antibody neutralizes the sporozoite stage of different Theileria parva stocks. *Parasite Immunol.*, 6: 361.
13. Dixit, A.K., Yadav, S.C. and Sharma, R.L. (2002), 28 kDa Fasciola gigantica cysteine proteinase in the diagnosis pre-patent ovine fasciolosis.*Vet. Parasitol*., 109: 233-247.
14. Dixit, A.K., Yadav, S.C., Saini, M. and Sharma, R.L. (2003): Purification and characterization of 28 kDa cysteine proteinase for immunodiagnosis of tropical fasciolosis. *J. Vet. Parasitol.* 17: 5-9.
15. Dixit, A.K., Yadav, S.C. and Sharma, R.L. (2004), Experimental bubalian fasciolosis: Kinetics of antibody response using 28 kDa Fasciola gigantica cysteine proteinase as antigen. *Trop. Anim. Health Prod*., 36: 49-54.
16. Dixit, A.K., Dixit, Pooja and Sharma, R.L. (2008), Immunodiagnostic/protective role of Cathepsin L cysteine proteinases secreted by Fasciola spp. *Vet. Parasitol.*, 154: 177-184.
17. Dobbelaere, D.A.E., Shapiro, S. Z. and Webster, P. (1985), Identification of a surface antigen on Theileria parva sporozoites by monoclonal antibody. *Proc. Natl. Acad. Sci.*, 82:1771.

18. Fagbemi, B.O. (1995), Development and characterization of monoclonal antibody reactive with a 28 kDa protease of Fasciola gigantica. *Vet. Parasitol*., 57: 351-356.
19. Fagbemi, B.O. and Hillyer, G.V. (1992), The Purification and characterization of a cysteine protease of Fasciola gigantica adult worms. *Vet. Parasitol*., 43: 223-232.
20. Gamble, H. R. and Graham, C. E. (1983), Comparison of monoclonal antibody based competitive and indirect enzyme linked immunosorbent assay for the diagnosis of swine trichinosis. *Vet. Immunol. Immunopathol.*, 6:379-389.
21. Gamble, H. R. and Graham, C. E. (1984), Monoclonal antibody-purified antigen for the immunodiagnosis of trichinosis. *Am. J. Vet. Res.*, 45: 67.
22. Gamble, H.R., Anderson, W.R., Graham, C.E. and Murell, K.D. (1983), Diagnosis of swine trichinosis by enzyme linked immunosorbent assay (ELISA) using an excretory-secretory antigen. *Vet. Parasitol.*, 13:349-361.
23. Grams, R., Vichasri –Grams, S., Sobhon, P., Upatham, E.S. and Viyanant, V. (2001), Molecular closing and characterization of cathepsin L encoding genes from Fasciola gigantica. *Parasitol. Int.*, 50: 105-114.
24. Handman, E.J., Goding, W. and Remington, J.S. (1980), Detection and characterization of membrane antigens of Toxoplasma gondii. *J. Immunol*., 124: 2578.
25. Hanna, R.E.B. and Trudgett, A.G. (1983), Fasciola hepatica: Development of monoclonal antibodies and their use to characterize a glycocalyx antigen in migrating flukes. *Parasite Immunol*., 5: 409-425.
26. Hanna, R.E.B., Anderson, A. and Trudgett, A. (1988), Fasciola hepatica: Studies on vaccination of rats and mice with a surface antigen prepared from fluke homogenate by means of a monoclonal antibody. *Res. Vet. Sci*., 44: 237-241.
27. Inthakanok L. (2007), Production and characterization of monoclonal antibodies against recombinant Cathepsin-L-A (rFgcatL-A) of Fasciola gigantica. M. Sc. (Environmental biology), Mahidol University.
28. Johnson, A. M., et. al.(1981), Hybridomas secreting monoclonal antibody to Toxoplasma gondii. *Aust. J. Biol. Exp. Med*., 59:303.
29. Johnson, A.M., McDonald, P.J. and Neoh, S.H. (1983), Monoclonal antibodies to Toxoplasma cell membrane surface antigens protect mice from toxoplasmosis. *J. Protozool*., 30:351.
30. Kaspar, L.H., Crabb, J.H. and Pfefferkorn, E.R. (1983), Purification of a major membrane protein of Toxoplasma gondii by immunoabsorption with a monodonal antibody. *J. lmmunol.*, 130: 2407.
31. Kaspar, L.H., Currie, K.M. and Bradley, M.S. (1985), An unexpected response to vaccination with a purified major membrane tachyzoite antigen (P30) of Toxoplasma gondii. *J. Immunol.*, 134: 3426.
32. Khaled, M. A. Q., Al-Omari, M.M., Al-Aghbar, M. and Abdel-Hafeez, S.K. (2008), Production of monoclonal antibodies against 8 kDa subunit of Echinococcus granulosus Antigen B (EgAgB8/2) using DNA immunization. *Hybridoma*, 27(6):431-438.
33. Liao, M., et. al. (2005), Identification and characterization of cross-reactive antigens from Neospora caninum and *Toxoplasma gondii*. *Parasitology*, 130(5): 481-488.
34. Mezo, M., Gonzalez-Warleta, M., Carro, C. and Ubeira, F.M. (2004), An ultrasensitive capture ELISA for detection of Fasciola hepatica coproantigens in sheep and cattle using a new monoclonal antibody (MM3). *J. Parasitol*., 90(4):845-52.
35. Molloy, J.B., et. al. (1998), Development of an enzyme linked immunosorbent assay for detection of antibodies to Babesia bigemina in cattle. *Parasitology*, 84(8): 651-656.
36. Musoke, A. J., Nantulya, V. M., Rurangirwa, F. R. and Buscher, G. (1984), Evidence for a common antigenic determinant on sporozoites of several *Theilieria parva* strains. *Immunology*, 52: 231.
37. Nakagaki K., Nogami, S., Hayashi, Y., Hammerberg, B., Tanaka, H. and Ohishi, I. (1993), Dirofilaria immitis: Detection of parasite-specific antigen by monoclonal antibodies in glomerulonephritis in infected dogs. *Parasitol. Res*., 79:49-54.
38. Ortega-Pierres, MacKenzie, G. C. D. and Parkhouse, R.M.E. (2007), Protection against Trichinella spiralis induced by a monoclonal antibody that promotes killing of newborn larvae by granulocytes. *Parasite Immunol.*, 6: 275.
39. Perryman L.E., Riggs, M.W., Mason, P.H. and Fayer, R. (1990), Kinetics of Cryptosporidium parvum sporozoite neturalization by monoclonal antibodies, immune bovine serum, and immune bovine colostrum. *Infect. Immun.*, 58: 257–9.
40. Raina, O.K., Yadav, S.C., Sriveny, D. and Gupta, S.C. (2006), Immuno-diagnosis of bubaline fasciolosis with Fasciola gigantica cathepsin-L and recombinant cathepsin L 1-D proteases.*Acta Trop*., 98 (2):145-151.
41. Sethi, K.K., Endo, T. and Brandis, H. (1980), Hybridomas secreting monoclonal antibody with specificity for *Toxoplasma gondii*. *J. Parasitol*., 66:192.
42. Sharma, S.D., Araujo, F.G. and Remington, J.S. (1984), Toxoplasma antigen isolated by affinity chromatography with monoclonal antibody protects mice against lethal infection with *Toxoplasma gondii*. *J. lmmunol.*, 133:2818.
43. Smith, H.V. and Rose, J.B. (1990), Waterborne cryptosporidiosis. *Parasitol. Today*, 6:8.
44. Solano, M., Ridley, R. K. and Minocha, H. C. (1991), Production and characterization of monoclonal antibodies against excretory – secretory products of Fasciola hepatica. *Vet. Parasitol*., 40: 227-239.
45. Sterling C.R. and Arrowood, M.J. (1986), Detection of Cryptosporidium sp.Infections using a direct immuno-fluorescent assay. Pediatr. *Infect. Dis.*, 5:139–42.
46. Tilley, M., Fayer, R., Guidry, S., Upton, S.J. and Blagburn, B.O. (1991), Antigens of oocysts and sporozoites of Cryptosporidium parvum (Apicomplexa: Crypto-sporidiidae) recognized by bovine colostral antibodies. *Infect. Immun*., 58:2966–71.
47. Tort, J., Brindley, P.J., Knox, D., Wolfe, K.H. and Dalton, J.P. (1999), Proteinases and associated genes of parasitic helminths. *Adv. Parasitol*., 43: 161-266.
48. Valero, M. A., et. al.(2008), MM3-ELISA evaluation of coproantigen release and serum antibody production in sheep experimentally infected with Fasciola hepatica and F. gigantica. *Vet. Parasitol.*, 159(1):77-81.
49. Wakelin, D. and Denham, D. A. (1983), The immune response. In: W. C. Campbell (Ed.). Trichinella and Trichinosis, Plenum Press, New York, pp 265-308.
50. Weil, G.J., Malane, M.S., Powers, K. G. and Blair, L.S. (1985), Monoclonal antibodies to parasite antigens found in the serum of Dirofilaria immitis-infected dogs. *J. Immunol.*, 134 (2): 1185-1191.
51. Wright, I. G., et. al. (1985), Protective vaccination against virulent Babesia bovis with a low molecular weight antigen. *Infect. Immun*., 48: 109.
52. Wright, I. G., et. al.(1983), Babesia bovis: Isolation of a protective antigen using monoclonal antibodies. *Infect. Immun.*, 41:244.