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Research Article

SDS PAGE Profile of *Raillietina tetragona* and *Raillietina cesticillus* of Domestic Fowl (*Gallus gallus domesticus*) for Development of Immunodiagnosis for Cestodiasis

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Abstract

A study was conducted to analyse the SDS PAGE profile of the excretory- secretory products and surface antigens of two cestode species- *Raillietina tetragona* and *Raillietina cesticillus* of domestic fowl (*Gallus gallus domesticus*) for development of immunodiagnosis for cestodiasis. The SDS-PAGE profile of the extracted proteins of the two cestode parasites, *Raillietina tetragona* and *Raillietina cesticillus*, of fowl resulted in total nine bands with molecular weights near to 150 kDa, 50 kDa, 37 kDa, 25 kDa, 20 kDa, 15 kDa, two thin bands lie between 37 kDa and 25 kDa and one band lies between 25 kDa and 20 kDa. The two parasites showed similar pattern with the difference that the 50 kDa band is thicker in the SDS-PAGE profile of *R. tetragona* than in the profile of *R. cesticillus,* which might be due structural differences between the proteins of the two parasites and may be used as a marker to differentiate the two species of the common genus. Further, cestodiasis in fowl has not yet received any attention with regards to immunodiagnosis. Thus, the present work attempted to analyse the soluble antigens of the two cestode parasites with the aim that the proteins would be identified and processed further for developing protocols for immunodiagnosis for cestodiasis in fowl.

Keywords: *Antigens; Cestodiasis; Immunodiagnosis; Raillietina tetragona; Raillietina cesticillus*

Introduction

Raillietina tetragona and *Raillietina cesticillus* belong to cestodes which causes cestodiasis in animals including domestic fowl (*Gallus gallus domesticus*). The cestode inhabits the small intestine and causes stunted growth of young chicken, emaciation of the adult, and decreased egg production of the hen [1]. The resultant situation leads to loss of body weight, retarded growth, reduced egg production, weakened body resistance and even death of the host [2]. In *R. tetragona* infection, the intestinal wall of the host intestine is thrown into ridges of purplish colour and the intestinal mucosa sloughs off. *Raillietina cesticillus* is a common tapeworm found in the jejunum of chickens and causes degenerative and inflammatory changes in the intestinal villi. The level of glucose and haemoglobin falls below normal level in the infected birds causing body malfunctions [3].

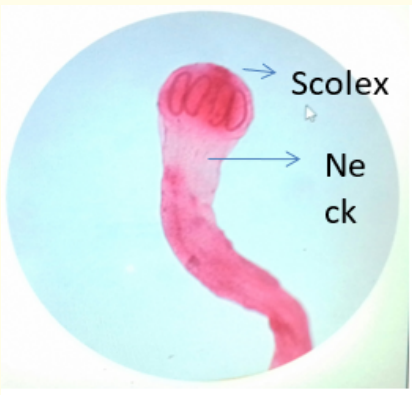

Figure 1: R. tetragona.

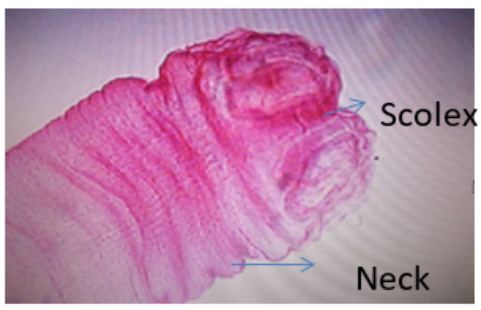

Figure 2: R. cesticillus.

Immunodiagnosis

The immunological techniques including ELISA, Western Blot, and SDS PAGE are employed to devise a protocol for early diagnosis without sacrificing hosts for gut examination. The antigens identified and purified in SDS PAGE profile are further used including devising diagnosis and vaccines in future.

Excretory secretory products and surface antigens of helminths (nematodes and cestodes)

Few decades ago the possibility that animals might acquire immunity to helminth parasites comparable to bacterial and viral infections was not acknowledged among helminthologists [4,5]. Aside from a preoccupation with taxonomy, life cycles and anthelmintics, a reason for this was the assumption that acquisition of immunity was unlikely as helminths frequently infected adult animals. But once it was known that infection with helminths prevented super infection, and recovery from infection resulted in a greater or lesser acquired immunity to subsequent infections [6-9], a steadily increasing number of publications on the immune reactions of hosts to helminth parasites appeared. Although interest in this field is relatively recent, much of the current research can be traced to the original observations and hypotheses of Chandler, Sarles, Stoll and Taliaferro. Because of the generally held concept that helminths, unlike protozoa, were incapable of stimulating immunity, much of the early research work on helminths focused on the development of diagnostic tests [10].

As a logical outgrowth on initial studies on serological diagnosis, precipitin tests were employed by Schwartz [11] and Hektoen [12] to study biological relationships and by Canning [13] to demonstrate antigenic specificity of nematode tissue. Succeeding years saw an amplification of experimental work along the line already established and the formulation of basic concepts and hypotheses. Metazoan parasites were recognized to be suitable for immunological studies, and at the same time to confirm established immunological principles [4].

Differences in the immune response of helminths, in contrast to those occurring in most bacterial infections, soon became apparent. Antibodies in the sera of animals immunized by natural or experimental infections could be measured by conventional techniques, and in some cases the protective ability was demonstrable by passive immunization. Immunity to the continuous accumulation of parasites, i.e. acquired immunity or the resistance to reinfection as the consequence of pre-existing infection, has long been known to exist, e.g. the tapeworm, *Taenia saginata*, in humans (Brumpt, 1927). The presence of even a single parasite of this species protects against additional infection, although protection is lost when parasites already present have been expelled.

The mechanism of acquired immunity in infections with parasitic worms has been reviewed by Taliaferro [4] and Taylor [14]. One of the first indications is the failure of the number of adult worms to increase as the result of continued reinfection, followed by a reduction in the number of established adult worms. Other manifestations of immunity are also apparent, namely (i) inhibition of reproduction as shown by a decrease in egg output; (ii) a tendency toward growth retardation, indicated by an increase in time required to reach maturity and the smaller size of worms at maturity; (iii) a tendency toward complete inhibition of development beyond the fourth larval stage; (iv) the self-cure phenomenon; and (v) a spring-rise in faecal egg counts associated with a loss of immunity.

In a pioneer work Sarles [15] and Taliaferro and Sarles [16] found that larvae of *N. brasiliensis* administered to previously infected rats were trapped in the lungs and skin by *in vivo* precipitates similar to those formed *in vitro* when infective larvae were placed in serum of previously infected rats. Moreover, an apparent secondary cellular response was observed. They found that both the precipitates and the characteristic cellular response to be manifested after challenge of passively immunized rats. Although the first *in vivo* precipitin tests were reported in *N. brasiliensis* by Sarles and Taliaferro [17], it was not until the work of Sarles [15] with the *in vitro* test with infections of this nematode in rats that the study of the serology of these infections was extensively pursued. This test consisted of the formation of precipitates at the oral, anal and excretory pore openings of developmental forms of N. brasiliensis when they were placed in serum from previously infected animals, the precipitates apparently caused by the combination of antibodies formed to the secretions and excretions of the worms in the host animal reacting to infection. Sarles [15] set forth the hypothesis that "the formation of oral and intestinal precipitates precedes and appears responsible for the inhibition of the activity and development of the parasite. Using fluorescent antibody techniques, Jackson [18] later confirmed that these precipitates did contain antibodies.

Helminths are not known to produce toxins which elicit the formation of antibodies comparable to those encountered in some bacterial diseases, but such antigenic substances as the ex-sheathing fluid described by Rogers and Sommerville [19,20] and Sommerville [21] and the proteolytic enzyme and lipase reported by Thorson (1953, 1956) may be important as specific antigens. Bueding and co-workers [22] have demonstrated specific immunological differences in enzymes catalyzing identical reactions in both schistosomes and vertebrates, and have suggested that vaccination with "metabolic products" may one day be possible.

Immunodiagnosis of cestodes

The diagnosis of cestode parasites in livestock is of major economic importance and mainly involves detection of the adult parasite or their metacestodes. However, due to the chronic nature of the disease caused by these parasites and the location of the larval stage in various tissues, accurate and timely detection of the parasites is often difficult [23].

Detection of cestode infections in animals are important because several species are zoonotic, causing cysticercosis and hydatidosis in man and because of the economic losses incurred due to infections in livestock. Thus, timely and accurate diagnosis of the problem is essential for freeing the hosts from the parasites. Such diagnosis could be both in the final and intermediate hosts. In the final hosts, the adult parasite, its segments or eggs could be identified in live patients whereas in the intermediate host where the cysts are embedded in tissues, it is very difficult to locate the cysts and the parasites unless the animal is killed or slaughtered. As some of the parasites and cysts are commonly hidden in soft tissues such as muscles, post-mortem examination may also often miss the majority of the infections although the level of detection depends on the skill of the inspector [24].

Also in case of the fowl cestodes, the intermediate hosts are tiny insects which are more difficult to handle for detection of cestode cysts harbouring them. This results in continued transmission and maintenance of the infections and failure to control or prevent the problem. Moreover, the best test should be one which could detect the disease early in order to achieve complete resolution of the disease.

Almost all available immunodiagnostic techniques, including methods for detecting specific antibodies and circulating parasite antigens in serum or other body fluids, have been applied for diagnosing cestodiasis. However, all the tools developed to date are generally applicable for laboratory research purposes only. None of the available diagnostic tools, kits, or methods are generally accepted by clinical physicians. Nevertheless, such serological tools are potentially important for epidemiological studies, confirmation of infection status and treatment and the monitoring of control programs and efforts should be continued so that new essays for improved, practical diagnosis of echinococcosis are developed [25]. With the advancement of proteomics and genomics a lot of immunological and molecular techniques for the diagnosis of cestodes and metacestode parasites of veterinary importance have been developed. Immunological diagnosis of infection in animals is difficult due to multiple infections with different species and antigenic cross reactivity between related parasites Moreover, many of the immunological diagnostic tests show less sensitivity and are not generally accepted by clinical physicians. As compared to immunological techniques, most of the molecular methods have higher sensitivity and specificity, but due to the relative higher cost few are commercially available. Most of the immunological and molecular diagnostic tests developed to date are generally applicable for laboratory research purposes. The developments in genomic and proteomic analysis should be used for further understanding of parasite-animal host interactions with the objective of finding additional targets for diagnosis.

Many authors have carried out their work on immunodiagnostics and vaccination against helminths. However, they have worked on different hosts including rabbit, rat and sheep. Also, their work was focussed on nematodes. Hence present work was focussed on immunodiagnostics of cestodes in fowl.

Material and Methodology

Preparation of cestode soluble protein extracts

Freshly collected parasite samples, collected from the gut of an infected fowl, were washed with ice cold PBS several times so as to reduce muscle constriction of the scolex and suckers to avoid release of excretory secretory products and enzymes which otherwise may become attached to the surface of adult parasite as described by Morales and Espino, 2012. Protein extracts (of the two species in two separate sets of replicates)were obtained by homogenization of 2 - 3 parasites in 5 ml of ice cold lysis buffer (pH 7.2). Homogenization was carried out for 10 minutes at 1300 rpm taking pause of 2 minutes after every one minute of homogenization. To 5 ml of lysis buffer 200 µl of cocktail of protease inhibitors (Roche) was added. After homogenisation, the mixture was allowed to stand for half an hour on ice till the foam settled down. Samples were then sonicated for 10 minutes (pulse 10 lapse 30 and amplitude 70%) on ice and centrifuged at 14000 rpm for 20 minutes at 40°C. The supernatant protein fraction was concentrated by AMICON® ultra using YM-3 membrane (cutoff, 3 kDa) for 20 minutes at 14g. The protein content was determined by Nano Drop.

SDS-PAGE analysis (three replicates per parasite sample)

The method commonly used to separate proteins is called SDS-PAGE (or sodium dodecyl sulfate polyacrylamide gel electrophoresis). In this procedure, an electrical field moves proteins through a gel matrix. SDS-PAGE, like horizontal agarose gel electrophoresis, separates the molecule of interest (protein in this case) by size.

Procedure

15 ul of the protein sample was taken in a microfuge tube and was added with 5 ul of sodium diodecylsulphate (SDS) solution for disrupting hydrophobic interactions, hydrogen bonds and disulphide bonds. The sample was then heated in a heating block for 2 - 3 minutes at 85°C to denature the proteins further. The SDS besides denaturing gives net negative charge to the proteins. 5 ul of Loading dye was added to the sample for visualization and increasing the density of proteins. Using P-20, 15 ul of sample was loaded into the wells along with ladder solution which acts as marker. The gel was allowed to run for 1-2 hours. The gel was then separated and soaked in Coomassie brilliant blue (CBB) to make the protein bands visible. Excess dye was removed by destaining with fixative solution.

Results

For immunodiagnostics immunological techniques are employed to design immunodiagnostics for the disease. In present study the SDS-PAGE analysis of harnessed cestode proteins of two cestode species- *R. tetragona* and *R. cesticillus* was done and the results are given below.

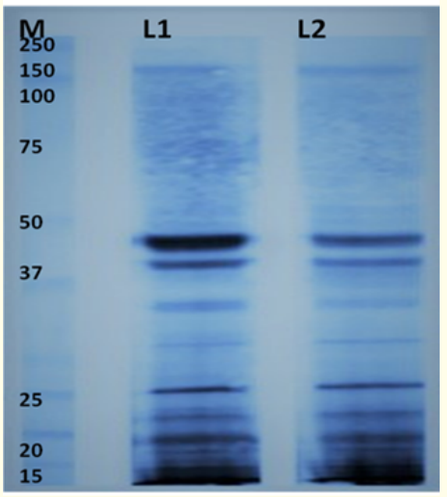

Figure 3: SDS PAGE profile of *R. tetragona* and *R. cesticillus*. Lane M shows ladder, Lane 1 shows proteins of *R. tetragona* and Lane 2 shows proteins of *R. cesticillus.*

The SDS-PAGE of the extracted proteins of the two cestode parasites of fowl results in total nine bands with molecular weights near to 150 kDa, 50 kDa, 37 kDa, 25 kDa, 20 kDa, 15 kDa, two thin bands lie between 37 kDa and 25 kDa and one band lies between 25 kDa and 20 kDa. The two parasites showed similar pattern with difference that the 50 kDa band is thicker in Lane1 than Lane2, which might be due to a structural difference between the two proteins of the parasites and can be used as a marker to differentiate the two species of the common genus.

Discussion

In the present work the SDS-PAGE page profile of the extracted proteins of the two cestode parasites *R. tetragona* and *R. cesticillus* of fowl resulted in total nine bands with molecular weights near to 150 kDa, 50 kDa, 37 kDa, 25 kDa, 20 kDa, 15 kDa, two thin bands lie between 37 kDa and 25 kDa and one band lies between 25 kDa and 20 kDa. The two parasites showed a similar pattern with difference that the 50 kDa band is thicker in Lane1 than in Lane2, which might be due to structural difference between the two proteins of the parasites and can be used as a marker to differentiate the two species of the common genus.

Youssefi., *et al*. [26] conducted a research work for determination of the electrophoretic pattern of somatic and excretory-secretory proteins of cestode parasites of *Ligula intestinalis* parasite in spirlin (*Alburnoides bipunctatus*) which showed5 protein bands of 26 kDa, 33 kDa, 38 kDa, 58 kDa and 70 kDa in somatic antigens, and 7 bands of 25, 28, 33, 43, 49, 60 and 70 kDa in excretory-secretory antigens. The patterns found show some similarities with the pattern of bands of the SDS-PAGE page profile of the extracted proteins of the two cestode parasites *R. tetragona* and *R. cesticillus* of fowl in the present study. Except the 150 kDa band found in the present study all other bands found in both the studies fall between 70 kDa to 15 kDa range.

Further the cestodiasis in fowl has not yet received any attention with regarding to immunodiagnosis. Thus, the present work attempted to analyse the soluble antigens of cestode parasites with aim that the proteins would be identified and processed further for developing protocols for immunodiagnosis for cestodiasis in fowl.

Conclusion

The present work was carried out to analyse the SDS-PAGE profile of the extracted soluble proteins of the two cestode parasites *R. tetragona* and *R. cesticillus* of fowl with hope that these proteins may be separated and identified and exploited for developing proper immunodiagnosis for cestodiasis of domestic fowl, *Gallus gallus domestics*, which is need of the hour. Moreover, the findings in this study gave us an insight into presence of 9 protein bands in crude worm extracts of *R. tetragona* and *R. cesticillus* that may evoke the host immune response by releasing antibodies against the worms. Further studies are required to determine exact information about the nature and function of the said proteins.

Bibliography

1. McDougald LR. "Cestodes and trematodes: Diseases of Poultry". 11th edition Blackwell Publishing Company: Iowa (USA) (2003): 961-72.

2. Hayat B and Hayat CS. "Incidence of intestinal parasites of chicken in Faisalabad district". *Pakistan Veterinary Journal* 3 (1983): 165-167.

3. Singh CDN., *et al*. "Advanced Pathology and Treatment of diseases of poultry with special reference to etiology, signs, pathology and management". 1st edition International Book Distributing Co., (2006): 99-102.

4. Taliaferro WH. "The mechanism of acquired immunity in infections with parasitic worms". *Physiological Reviews* 20.4 (1940): 469-492.

5. Urquhart GM., *et al*. "Helminth immunity". In CA Brandley and EL Jungherr, eds. Advances in Veterinary Science, Volume 7, Academic Press, New York (1962): 87-129.

6. Africa CM. "Studies on the host relations of Nippostrongylus muris, with special reference to age resistance and acquired immunity". *Journal of Parasitology* 18.1 (1931): 1-13.

7. Chandler AC. "Experiments on resistance of rats to super infection with the nematode, Nippostrongylus muris". *American Journal of Hygiene* 16.3 (1932): 750-782.

8. Miller HM and Gardiner ML. "Further studies on passive immunity to a metazoan parasite, Cystieereus faseiolaris". *American Journal of Hygiene* 20.2 (1934): 424-431.

9. Schwartz B., *et al*. "Resistance of rats to super infection with a nematode Nippostrongylus muris and an apparently similar resistance of horses to super infection with nematodes". *Journal of the Washington Academy of Sciences* 21.12 (1931): 259-261.

10. Tromba FG. "Immunology of nematode diseases". *Parasitology* 48.6 (1962): 839-845.

11. Schwartz B. "The biological relationships of ascarids". *Journal of Parasitology* 6.3 (1920): 115-123.

12. Hektoen L. "The precipitin reactions of extracts of various animal parasites". *Journal of Infectious Diseases* 39.4 (1926): 342-344.

13. Canning GA. "Precipitin reactions with various tissues of Ascaria lumbricoides and related helminths". *American Journal of Hygiene* 9.1 (1929): 207-226.

14. Taylor E. "An account of the gain and loss of the infective larvae of parasitic nematodes in pastures". *Veto Research* 69 (1957): 557-563.

15. Sarles MP. "The in vitro action of immune cat serum on the nematode, Nippostrongylus muris". *Journal of Infectious Diseases* 62 (1938): 337-348.

16. Taliaferro WH and Sarles MP. "Cellular reaction during, immunity to Nippostrongylus muris in the rat". *Journal of Parasitology* 23 (1937): 561.

17. Sarles MP and Taliaferro WH. "The local points of defense and the passive transfer of accquired immunity to Nippostrongylus muris in rats". *Journal of Infectious Diseases* 59.2 (1936): 207-220.

18. Jackson GJ. "Fluorescent antibody studies of Nippostrongylus muris infections". *Journal of Infectious Diseases* 106 (1960): 20-36.

19. Rogers WP and Sommerville RI. "Physiology of exsheathment in nematodes and its relation to parasitism". *Nature* 179.4560 (1957): 619-621.

20. Rogers WP and Sommerville RI. "The physiology of the second ecdysis of parasitic nematodes". *Parasitology* 50 (1960): 329-348.

21. Sommerville Rl. "Histotrophic phase of the nematode parasite, Os/ertagia circumcincta". *Australian Journal of Agricultural Research* 5.1 (1954): 130-140.

22. Bueding E and Mackinnon A. "Hexokinases of Schislosoma manso ni". *BioChem* 215 (1955): 495-506.

23. Alemu S., *et al*. "Immunological and Molecular Diagnostic Tests for Cestodes and Metacestodes Review". *World Applied Sciences Journal* 33.12 (2015): 1867-1879.

24. Lightowlers MW. "Cestode infections in animals: immunological diagnosis and vaccination". *Revue Scientifique et technique (International Office of Epizootics)* 9.2 (1990): 463-487.

25. Zhang W., *et al*. "Review Article on Immunology and Immunodiagnosis of Cystic Echinococcosis: An Update". *Clinical and Developmental Immunology* (2012): 101895.

26. Youssefi MR., *et al*. "Determination of the electrophoretic pattern of somatic and excretory-secretory proteins of Ligula intestinalis parasite in spirlin (Alburnoides bipunctatus)". *Tropical Biomedicine* 29.4 (2012): 519-523.

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