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Immunogenic Proteins Characterization in the Larvae of *Oestrus ovis* by Western Blot Analysis

A. Vamshi Kiran [a++*], G.S. Sreenivasa Murthy [b#], M. Udaya Kumar [a†] and P. Kalyani [c‡]

[a] Department of Veterinary Parasitology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana 500030, India.
[b] Department of Veterinary Parasitology, College of Veterinary Science, Korutla, Jagtial, 505326, India.
[c] Department of Veterinary Biotechnology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana 500030, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was undertaken to characterize and identify immunogenic proteins of *Oestrus ovis* in Telangana. *Oestrus ovis* is the most predominant nasal bot fly causing nasal myiasis in India. The collected larvae from Jiyaguda slaughter house, Hyderabad are seperated into L_2 and L_3

[++] M.V.Sc Student;
[#] Professor and University Head;
[†] Associate Dean and Professor;
[‡] Head and Assistant Professor;
**Corresponding author: E-mail: vamshikiran181@gmail.com;*

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larvae were washed with normal saline and PBS pH 7.4 and preserved at -70°C for antigen preparation and some were preserved in 70 % ethanol for identification. The larvae were speciated by light microscopic examination based on morphological features such as ventral spines, anterior hooks and "D" shaped posterior spiracles. In this study, an antigen was prepared from second and third instar larvae of *Oestrus ovis* using a soluble buffer solution. The resulting Crude Somatic Larval (CSL) antigen had a protein concentration of 27.4 mg per ml. The CSL antigen was resolved in 8% SDS-PAGE, Immunoblotting was performed by electro transferring the separated polypeptides onto a nitrocellulose (NC) membrane. Treatment with specific reagents and antibodies resulted in the identification of 19 immunoreactive polypeptides, with molecular weights matching those observed in SDS-PAGE. Four polypeptides with molecular weights of 58.56, 20.74, 19.09, and 28.91 kDa exhibited the strongest reaction towards the antibodies raised against CSL antigen. These four polypeptides are considered promising candidates for future immunizations and testing their protective efficacy through in vivo assays. These findings have implications for advancing strategies to combat myiasis, a parasitic disease caused by *Oestrus ovis* larvae.

Keywords: Double immuno diffusion; nasal bot fly; Oestrus ovis; SDS-PAGE; crude somatic larval antigen; immune polypeptides; western blotting; myiasis; agar gel precipitation.

1. INTRODUCTION

Oestrus ovis, also known as the nasal bot fly, is a type of fly that parasitizes different animals like sheep, goats, and even humans. These parasites are particularly known for affecting livestock and, sometimes, causing infections in humans. The larvae of *Oestrus ovis* are obligatory parasites. *Oestrus ovis* larval instars (L2 and L3) and adult flies that developed from mature larvae were identified based on stigmal plates and morphological characteristics [1]. They have been extensively researched in various studies worldwide, showing up in many countries due to their wide distribution [2,3,4].

The larvae in infected sheep can cause histopathological changes in nasal tissues, allergic and inflammatory responses, followed by bacterial infection and sometimes death. Pathophysiological changes caused by *Oestrus ovis* in small ruminants [5]. The immunological studies conducted for diagnosing *Oestrus ovis* infections in sheep have primarily relied on using larval extracts as antigen sources in tests like indirect hemagglutination and enzyme-linked immunosorbent assay (ELISA). While specific antibodies have been detected in infested sheep, there is a need to further explore the tissue specificity of these antibodies, as outlined by Bautista et al. [6]. Characterizing protective immunogens, with particular attention to a 28 kDa protein complex (pc28), recognized as a key antigenic component present in excretory and secretory products derived from *Oestrus ovis* larvae, as documented by Tabouret et al. [7]. These findings underscore the critical importance of unraveling the immunological aspects of *Oestrus ovis* infestations, as this knowledge serves as a cornerstone in the development of effective vaccines, not only in India but also in other regions. Hence, the present investigation was planned to identify *Oestrus ovis* in Telangana, India and characterize the protective antigens of larvae, which can help future vaccine development research.

2. MATERIALS AND METHODS

The second and third instar larvae homogenized adequately using lysis buffer containing 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 7.5, 1 mM PMSF (dissolved in Propanol) & 6 mM urea at 4 ºC until a homogenous suspension was formed. Centrifuged at 14,000 rpm for 15 min at 4 ºC. The supernatant was collected and preserved at -70 ºC. The antigen was prepared according to Innocenti et al. [8]. The protein content of supernatant of crude somatic larval antigen sample was estimated by Lowry method [9] in a nanodrop spectrophotometer (Thermo Fisher Scientific).

The supernatant of crude somatic pooled L_2 and L_3 antigen was resolved in 8% gel by SDS-PAGE (under reducing conditions) in a discontinuous buffer system to study the protein profile for determining the number of proteins and their molecular weights based on relative mobility values. The SDS-PAGE was run in 8% separating gel and 4 % stacking gel (discontinuous buffer system) in GeNei Small Vertical Gel Electrophoresis System.

In this study, two New Zealand white rabbits were employed to generate antibodies against

the crude somatic pooled L_2 and L_3 antigens of Oestrus ovis. Initially, the rabbits received injections of the antigens combined with Freund's Complete Adjuvant, followed by four booster doses with Freund's Incomplete Adjuvant at weekly intervals. Blood samples were collected from the marginal ear vein on the 27th day post-first injection to assess antibody response using agar gel precipitation tests. Further blood samples were obtained via cardiac puncture on the sixth day post-fourth booster injection. Serum containing antibodies against the antigen was isolated, underwent complement inactivation, and was subsequently tested for antibody presence using Double Immuno Diffusion (DID).

The sera containing the desired antibodies were aliquoted and stored at -20 ºC for future assays. Western blotting was conducted to detect immunogenic polypeptides from *Oestrus ovis* L_2 and L_3 stage. The electro-transfer of resolved polypeptides in 8 % SDS-PAGE gel onto nitrocellulose (NC) membrane was conducted in a GeNei Electrotransfer Mini System Gel Size (8 cm x 7 cm).

3. RESULTS

Double Immuno Diffusion (DID) test: Hyper immune serum raised in rabbit against *Oestrus ovis* crude somatic pooled L2 and L3 antigen was tested by Double Immune Diffusion (DID) to know whether antibodies developed against CSL antigen or not. The test revealed a clear precipitin band after 24 hrs of incubation indicating the presence of antibodies in the serum raised against CSL antigen.

Western blotting analysis of SDS-PAGE of CSL Ag: The separated polypeptides were transferred successfully onto the nitrocellulose membrane. The transferred polypeptides on NC membrane were checked for their immunoreactivity to hyperimmune serum by western blotting analysis. The polypeptides of CSL resolved on 8 % SDS-PAGE and transferred on to the NC membrane were kept for blocking with skim milk for 1 hour and reacted well with HIS at 1:200 dilution for 2 hours on rocker followed by Goat anti rabbit IgG HRP at 1:2000 dilution at room temperature for 1 hr and yielded 19 polypeptides of 58.56, 51.71, 48.58, 44.71, 43.79, 42.01, 40.30, 37.09, 35.58, 31.4, 28.91, 27.74, 24.49, 23.49, 20.74, 19.09, 17.57, 16.17 and 14.27 kDa, molecular weights. Out of 19 immunoreactive polypeptides, 9 polypeptides (58.56, 48.58, 42.01, 40.30, 28.91, 27.74, 20.74, 19.09 and 14.27 kDa) were of high intensity, of which 58.56 kDa polypeptide showed most intense reaction to HIS and remaining 10 polypeptides (51.71, 44.71, 43.79, 37.09, 35.58, 31.4, 24.49, 23.49, 17.57, 16.17 kDa) were of moderate intensity.

Fig. 1. Photograph shows precipitation band of CSL Ag with hyperimmune serum in Double Immuno Diffusion test

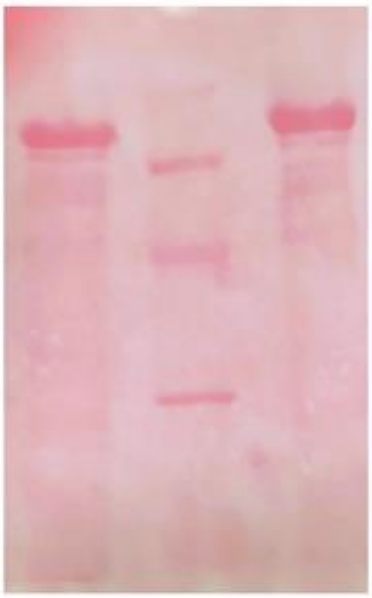

Fig. 2. Photograph showing protein transferred on to NC membrane in Ponceau stain

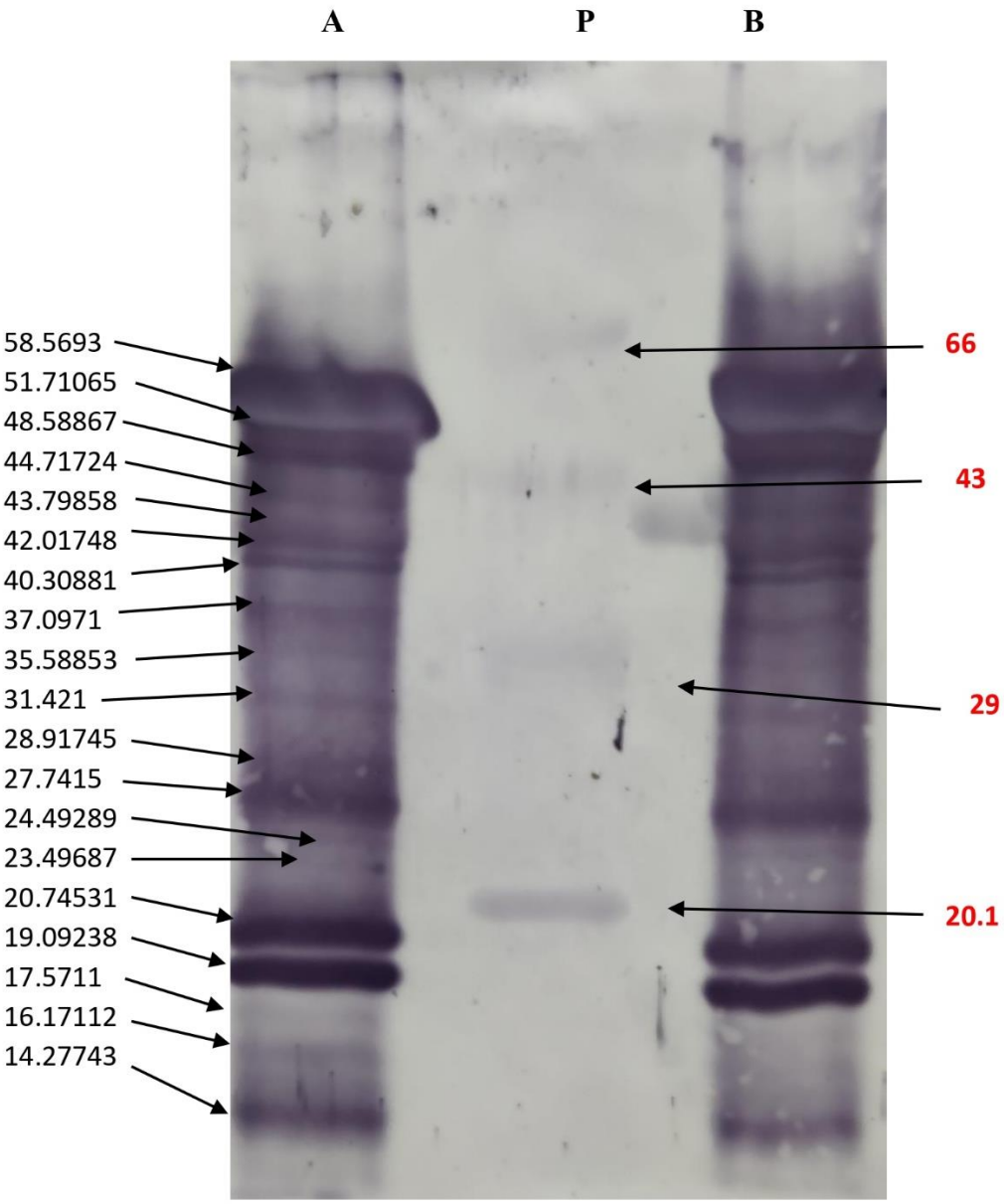

Fig. 3. Photograph showing western blot analysis of CSL Ag of *Oestrus ovis*
LANE A - Immunoreactivity of CSL polypeptides in western blotting analysis
P – Protein marker
A – Molecular weights of immunoreactive polypeptides
LANE B – Immunoreactivity of CSL polypeptides in western blotting analysis

4. DISCUSSION

The crude somatic larval (CSL) antigen was used for the western blotting to understand the antigenicity of various antigenic proteins present in this extract. In the present study, the NC membrane blotted strip was incubated with antiserum raised against CSL antigen at 1:200 dilution for 2 hours on rocker, followed by Goat anti rabbit IgG HRP at 1:2000 dilution for 1 hr at room temperature. These dilutions were optimum for standardized immunoblot assay and were able to get 19 immunoreactive polypeptides on immunoblot.

Out of 19 immunoreactive polypeptides, 9 polypeptides (58.56, 48.58, 42.01, 40.30, 28.91, 27.74, 20.74, 19.09 and 14.27 kDa) were of high intensity, of which 58.56 20.74 and 19.09 kDa polypeptide showed most intense reaction to

HIS. Remaining 10 polypeptides (51.71, 44.71, 43.79, 37.09, 35.58, 31.4, 24.49, 23.49, 17.57, 16.17 kDa) were moderately reactive.

Several electrophoretic analysis of proteins of *Oestrus ovis* larvae (L_2 and L_3) and immunoblot studies have been conducted with some similarities and some differences in the results of protein profiles and antigenic activity in immunoblotting tests.

In our study, the immunoreactive polypeptides with a molecular weight of 28.91 kDa align with the findings of Angulo-Valadez et al. [10]. Their research, conducted via electrophoresis under non-reducing conditions, also identified a protein pattern in *O.ovis* L_3 salivary gland products with the highest antigenic activity around the 29.0 kDa band.

Alborzi et al. [11] conducted a study in which they employed 8.0% acrylamide gel electrophoresis under reducing conditions to analyze the electrophoretic patterns of proteins and Excretory and secretory products (ESP) extracted from *O. ovis* larvae (L_2 and L_3). Their investigation revealed the presence of a diverse array of protein bands with molecular weights ranging from 79 to small peptides below 14.6 kDa. In subsequent immunoblotting tests, they identified specific protein bands at 58.0, 47.0, 42.0, 29.0, and 28.0 kDa in the proteins extracted from *O. ovis* larvae (L_2 and L_3) collected from the host animals. Notably, among the protein profiles, the 28.0 kDa protein emerged as the most prominent antigenic component. In our present study, we obtained results that align closely with the findings of Alborzi et al. [11] as we also identified proteins with molecular weights of 58.56, 48.58, 42.01, 28.91 and 27.74. These consistent results suggest a shared pattern in the antigenic components of *O. ovis* larvae between Alborzi et al. [11] study and present study.

Similarly, Arunkumar et al. [12] identified eight distinct protein bands in the excretory-secretory antigens of *Oestrus ovis* larvae, each with specific molecular weights. Our study also detected polypeptides at 63.63 kDa, 56.18 kDa, 40.30 kDa, 31.42 kDa, 23.49 kDa, and 16.17 kDa. These findings align closely with those of Arunkumar et al. [12], reinforcing the presence of these particular polypeptides in the excretory-secretory antigens of *Oestrus ovis* in both studies.

5. CONCLUSSION

It is concluded that, the present study identified 19 immunoreactive polypeptides from the second and third instar larvae of *Oestrus ovis*. Some of our findings align with those of previous researchers. These identified immunogenic proteins represent potential candidates for future vaccine development strategies in India aimed at safeguarding livestock against myiasis caused by *Oestrus ovis*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Permission was accorded by Ethical committee bearing Ref: 698/CPCSEA dated 01/10/2002 F. No. 25/60/2010- AWD/Veterinary college/ Hyderabad. IAEC approval No. for the project - 12/ 26/ C.V.Sc., Hyd. IAEC –Rabbits, dated 04/ 1/2023.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sree CJ, Sreedevi C, Samatha V. *Oestrus ovis* in sheep in Krishna District, Andhra Pradesh. Journal of Veterinary Parasitology; 2022.
2. Jagannath MS, Cozab N, Rahman SA, Honnappa TG. Factors in fluencing *Oestrus ovis* infestation in sheep and goats. Current Research. 1989;18:118-119.
3. Alahmed AM. Seasonal infestation of *Oestrus ovis* larvae in sheep heads in central region of Saudi Arabia. Journal of the Egyptian Society of Parasitology. 2000;30:895-901.

4. Abo-Shehada MN, Araba B, Mekbela R, Williams D, Torgerson PR. Age and seasonal variations in the prevalence of *Oestrus ovis* larvae among sheep in northern Jordan. Preventive Veterinary Medicine, 2000;47:205-212.
5. Saleem T, Katoch R, Yadav A, Godara R. Study on pathological changes causedby *Oestrus ovis* in small ruminants of Jammu. Indian Journal of Small Ruminants. 2017;23(1):52-55.
6. Bautista GCR, Angulo-Contreras RM, Garay-Garzon E. Serologic diagnosis of *Oestrus ovis* in naturally infested sheep. Medical and Veterinary Entomology. 1988;2:351-355.
7. Tabouret G, Prevot F, Bergeaud JP, Dorchies P, Jacquiet, P. *Oestrus ovis*: Sheep humoral immune response to purified excreted/secreted salivary gland 28 kDa antigen complex from second and third instar larvae. Veterinary Parasitology. 2001;101:53-66.
8. Innocenti L, Masetti M, Macchioni G and Giorgi F. Larval salivary gland proteins of the sheep nasal bot fly larva, are major immunogens in infested sheep. Veterinary Parasitology, 1995;60:273-282.
9. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry. 1951;19:265-275.
10. Angulo-Valadez CE, Cepeda-Palacios R, Jacquiet P, Dorchies P, Prevot F, Ascencio-Valle F, Torres F. Effects of immunization of pelibuey lambs with *Oestrus ovis* digestive tract protein extracts on larval establishment and development. Veterinary Parasitology. 2007;143:140-146.
11. Alborzi A, Jolodar A, Shapouri MS. Isolation and dentification of excretory-secretory and somatic antigens from the *Oestrus ovis* larvae by SDS-PAGE and immunoblotting. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. Veterinary Research Forum. 2014;5:307.
12. Arunkumar S, Krupakaran R P, Perumal, P. An analysis of matrix metalloproteinases (MMPs) activity in excretory/secretory antigens of *Oestrus ovis* larvae from sheep. Indian Journal of Animal Sciences. 2017;87(8):947-950.

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