Uttar Pradesh Journal of Zoology

Volume 45, Issue 16, Page 543-549, 2024; Article no.UPJOZ.3874
ISSN: 0256-971X (P)

Proteomic Characterization of *Oestrus ovis* Larvae by SDS Page 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-500030, Telangana, India.
[b] Department of Veterinary Parasitology, College of Veterinary Science, Korutla, Jagtial, 505326, Telangana, India.
[c] Department of Veterinary Biotechnology, College of Veterinary Science, Rajendranagar, Hyderabad-500030, Telangana, India.

Authors' contributions

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

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i164335

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3874

Original Research Article

Received: 02/06/2024
Accepted: 03/08/2024
Published: 08/08/2024

ABSTRACT

This study focuses on the proteomic characterization of crude somatic pooled L2 and L3 antigens from *Oestrus ovis* larvae. The larvae were homogenized in a buffer containing 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, 1 mM PMSF, and 6 mM urea, followed by centrifugation at 4 ºC to obtain soluble crude larval proteins. The protein concentration of the pooled crude somatic L2 and L3 antigen was determined to be 27.4 mg/ml using the Lowry method. From 26 second-stage and 13 third-stage larvae, 12 ml of antigen was obtained, aliquoted, and stored at -70 ºC. For protein profile analysis, SDS-PAGE was performed with varying concentrations of CSL proteins, revealing multiple polypeptides. Specifically, 50 µg of CSL protein loaded on SDS-PAGE resolved into 27 distinct

**Corresponding author: Email: vamshikiran181@gmail.com;*

Cite as: *Kiran, A. Vamshi, G. S. Sreenivasa Murthy, M. Udaya Kumar, and P. Kalyani. 2024. "Proteomic Characterization of Oestrus Ovis Larvae by SDS Page Analysis". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (16):543-49. https://doi.org/10.56557/upjoz/2024/v45i164335.*

polypeptides with molecular weights ranging from 14.27 kDa to 81.64 kDa. This diversity in molecular sizes underscores the complex nature of the CSL protein, indicating the presence of various protein components within the larval stages. These findings lay the groundwork for further investigations into the immunogenicity and potential applications of these proteins in the diagnosis, treatment, or management of nasal myiasis caused by *Oestrus ovis*.

Keywords: Oestrus ovis; nasal bot fly; myiasis; crude somatic larval peptides; SDS-PAGE.

1. INTRODUCTION

Oestrus ovis, commonly known as the nasal bot fly, is a parasitic dipteran species that infests various vertebrate animals, including sheep, goats, and occasionally humans. These parasites significantly impact livestock and sometimes cause zoonotic infections in humans. The larvae of *Oestrus ovis* are obligate parasites and have been extensively studied in various epidemiological investigations worldwide.

The economic impact of *Oestrus ovis* infestations is a primary concern. These parasitic larvae cause pathological changes in nasal tissues, leading to allergic and inflammatory responses, bacterial infections, and occasionally death. Adult flies contribute to annoyance and reduced productivity, resulting in substantial economic losses. These include reductions in weight gain (1 to 4.5 kg), wool production (200 to 500 g), and milk production (up to 10%). These economic losses are compounded by treatment costs [1,2].

The pathogenesis of *Oestrus ovis* infections is complex and multifactorial. It is partly due to mechanical trauma from larval movement within the host's nasal cavities and irritation caused by the cuticular spines and oral hooks. However, the primary cause of pathogenesis is the biomolecules, including enzymes and antigens, secreted by the larvae onto the mucosa, inducing a hypersensitivity immune reaction [3]. The severity of pathogenesis increases with larval development, especially with L3 larvae, due to an increase in protein production and proteolytic activity [4].

To mitigate the effects of *Oestrus ovis* myiasis and protect livestock, various control methods have been proposed. Some researchers recommend intranasal insecticides in aerosol form. However, prolonged use of insecticides can negatively affect the environment and may lead to resistant larval strains [5]. Consequently, a more biological approach to control has been introduced, focusing on controlling the reproductive potential of parasitic species. This approach includes attempts at vaccination against larval fly extracts [6-8].

Hence, the present investigation was planned to identify *Oestrus ovis* larvae in Telangana, India, and characterize the polypeptide profile of *Oestrus ovis* larvae on 8% SDS-PAGE. This characterization can be instrumental for future research.

2. MATERIALS AND METHODS

2.1 Preparation of Protein Sample

The second and third instar larvae, previously preserved at -70°C, were thawed and homogenized. This process involved utilizing a lysis buffer containing 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA at pH 7.5, and a combination of 1 mM PMSF (dissolved in Propanol) along with 6 mM urea at 4°C until achieving a uniform suspension. The suspension was then centrifuged at 2192 g for 15 minutes at 4°C. The supernatant, containing the desired protein components, was carefully collected and stored at -70°C. The protein sample preparation followed the methodology outlined by Innocenti et al. [9].

2.2 Estimation Protein Concentration in Sample

The protein content of supernatant of crude somatic L_3 sample supernatant was estimated using the Lowry method with a Nanodrop spectrophotometer (Thermo Fisher Scientific) [10].

2.3 SDS-PAGE (Sodium Dodecyl sulphate – Polyacrylamide Gel Electrophoresis)

The supernatant of crude somatic pooled L_2 and L_3 was resolved in 8% gel by SDS- PAGE in a discontinuous buffer system to study the protein profile, the molecular weights of peptides were calculated based on the migration of peptides which was compared to the logarithmic values of

a standard marker's molecular weights. The SDS-PAGE was run in 8% separating gel and 4% stacking gel in GeNei Small Vertical Gel Electrophoresis System.

3. RESULTS

3.1 Crude Somatic Pooled L2 and L3 Antigen

The whole second (L2) and third instar larvae (L3), preserved at -70ºC, were thawed and homogenized with 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA (pH 7.5), 1 mM PMSF (dissolved in propanol), and 6 mM urea. The homogenate was centrifuged at 4ºC to obtain soluble crude larval proteins. The pooled crude somatic L2 and L3 antigen was extracted, and the constituent proteins were characterized by SDS-PAGE analysis. Immunogenic proteins were further identified by western blotting.

3.2 Protein Estimation of CSL Antigen

The protein concentration of pooled crude somatic L_2 and L_3 antigen was determined to be 27.4 mg/ml using the Lowry method. The crude somatic larval (CSL) antigen was prepared using 26 second stage larvae and 13 third stage larvae of *Oestrus ovis,* which yielded 12 ml of antigen. The antigen was made into aliquots of 500 µL and stored at -70ºC for further use [10].

3.3 Sensitivity Analysis of SDS-PAGE – CSL Antigen

SDS-PAGE was performed by loading different gradient concentrations of CSL proteins starting from 10 µg to 100 µg/ well. A total of 20 µL CSL proteins, ranging from 10 µg to 100 µg per well. A total of 20 µL of CSL protein, along with Laemmli buffer, was loaded into each well. SDS-PAGE of 50 µg of CSL protein revealed multiple polypeptides (Figs. 1 and 2).

3.4 Protein Profile of CSL Antigen by SDS-PAGE

The crude somatic pooled L2 and L_3 antigen (CSL) at a standard concentration of 274 µg (10 µL) of *Oestrus ovis* was resolved on an 8% SDS-PAGE gel, alongside a standard protein molecular marker (Bangalore Genei), revealing 27 polypeptides (Fig. 3).

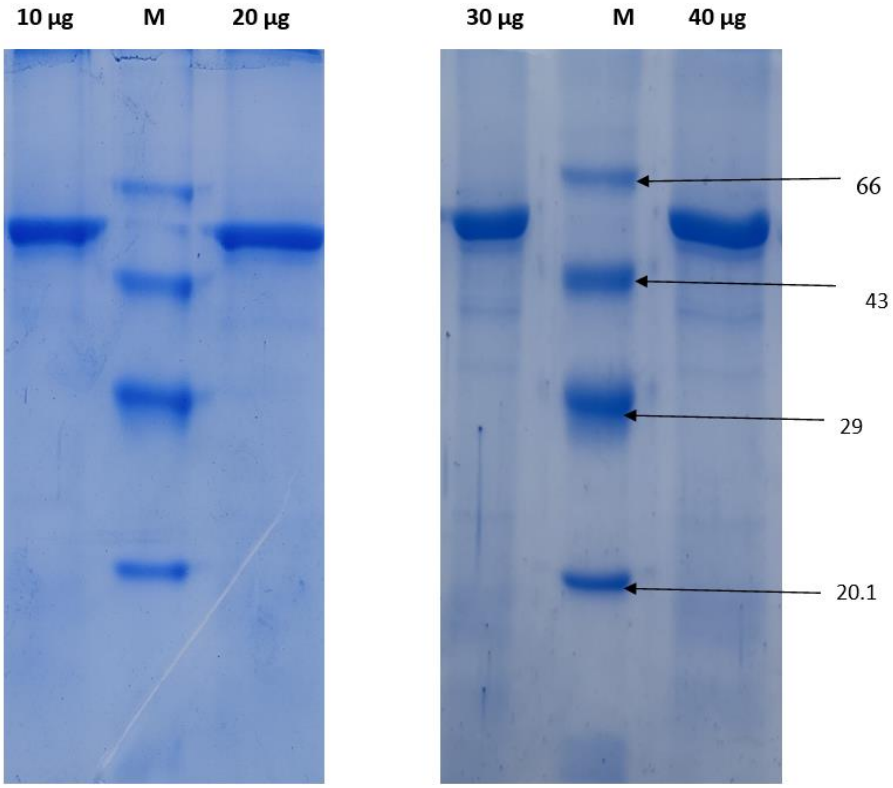

Fig. 1. Photograph showing of SDS-PAGE analysis of CSL Ag (concentration of 10- 40 µg) on 8% separating gel

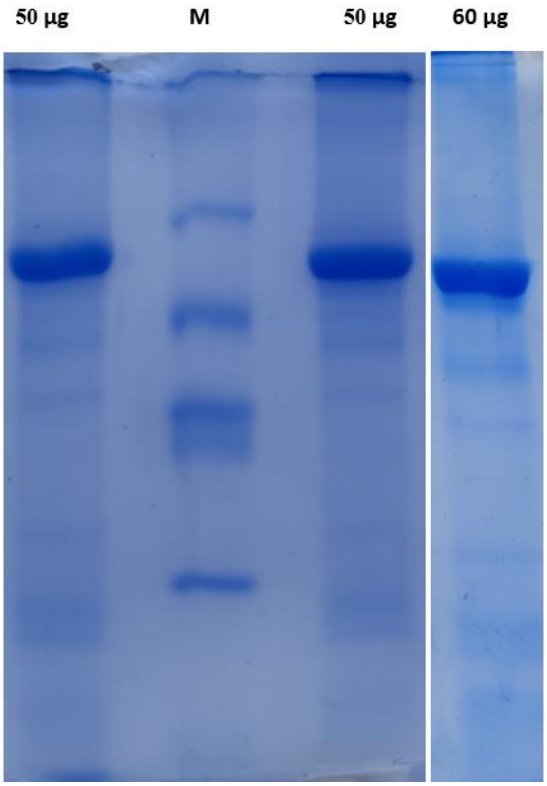

Fig. 2. Photograph showing sensitivity analysis of SDS-PAGE analysis of CSL Ag (concentration of 50- 60 µg) on 8% separating gel

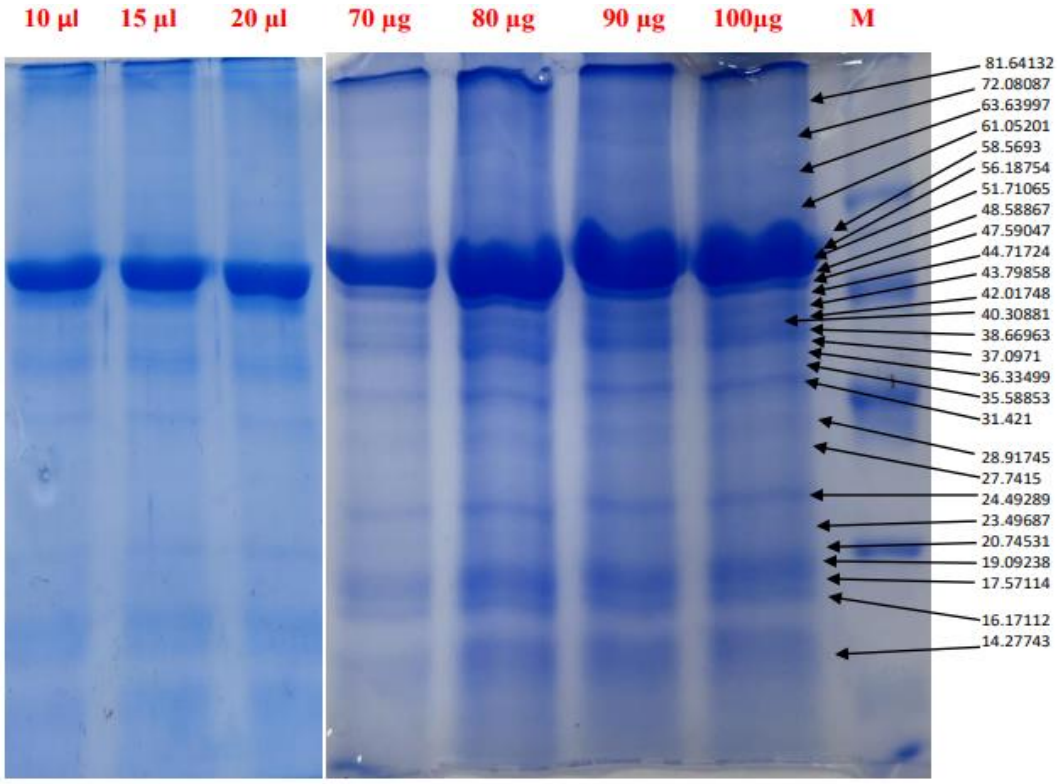

Fig. 3. Photograph showing of SDS-PAGE analysis of CSL protein (60 µg at 3 dilutions 10 µl ,15 µl and 20 µl and concentration 60-100 µg) on 8% separating gel

3.5 Determination of Molecular Weight of Resolved Crude Somatic Larval Antigen (CSL) Peptides

The molecular weights of peptides were calculated based on the migration of peptides which was compared to the logarithmic values of a standard marker's molecular weights (Fig. 4).

The standard curve for the log values of molecular weights revealed 27 polypeptides of CSL antigen on 8% SDS-PAGE, ranging from 81.64 kDa to 14.27 kDa (Table 1).

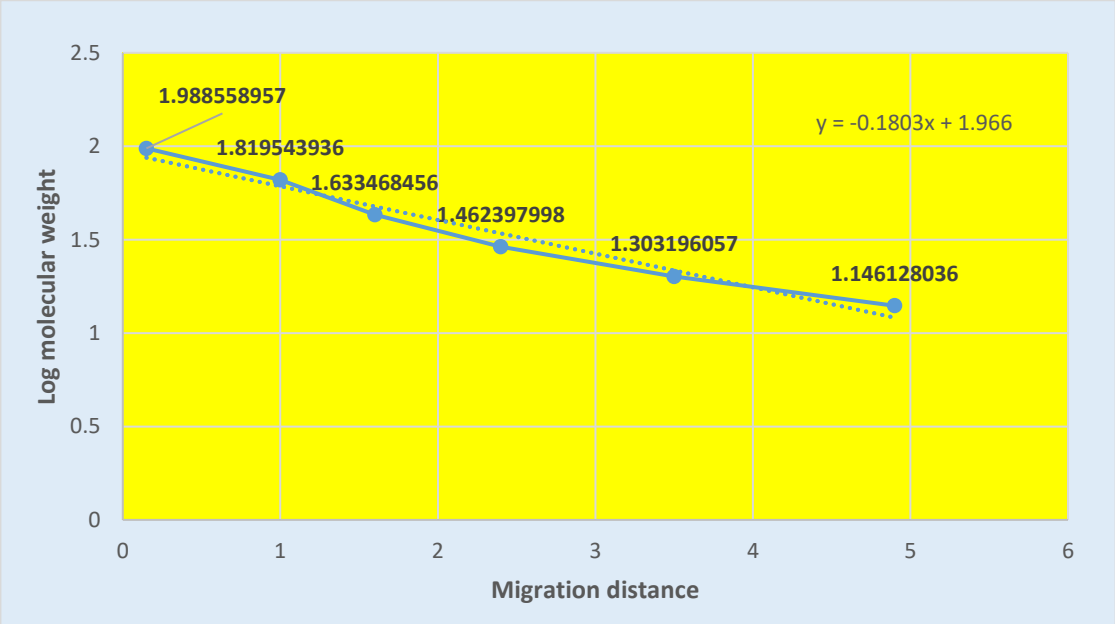

Fig. 4. Graph showing Standard protein curve for log values of molecular weight SDS-PAGE

Table 1. Migration distance of protein, log value and molecular weights of polypeptides separated on 8 % SDS-PAGE gel

S. No	Migration distance of protein	Log value of protein (=log(distance of protein))	Molecular weight(=10^log)
1.	0.3	1.91191	81.64132
2.	0.6	1.85782	72.08087
3.	0.9	1.80373	63.63997
4.	1	1.7857	61.05201
5.	1.1	1.76767	58.5693
6.	1.2	1.74964	56.18754
7.	1.4	1.71358	51.71065
8.	1.55	1.686535	48.58867
9.	1.6	1.67752	47.59047
10.	1.75	1.650475	44.71724
11.	1.8	1.64146	43.79858
12.	1.9	1.62343	42.01748
13.	2	1.6054	40.30881
14.	2.1	1.58737	38.66963
15.	2.2	1.56934	37.0971
16.	2.25	1.560325	36.33499
17.	2.3	1.55131	35.58853
18.	2.6	1.49722	31.421
19.	2.8	1.46116	28.91745
20.	2.9	1.44313	27.7415

S. No	Migration distance of protein	Log value of protein (=log(distance of protein))	Molecular weight(=10^log)
21.	3.2	1.38904	24.49289
22.	3.3	1.37101	23.49687
23.	3.6	1.31692	20.74531
24.	3.8	1.28086	19.09238
25.	4	1.2448	17.57114
26.	4.2	1.20874	16.17112
27.	4.5	1.15465	14.27743

4. DISCUSSION

In our study, SDS-PAGE was employed to examine the protein profile in the crude somatic larval (CSL) extract of *Oestrus ovis*. This extract contained a mix of 27 different polypeptides with sizes ranging from 14.27 to 81.64 kilodaltons (kDa).

Previous studies by Innocenti et al*.,* [11] identified specific protein fragments from the cuticle of third instar larvae of *Oestrus ovis*, including a 56.0 kDa fragment that strongly reacted with antibodies in sheep. Interestingly, in our current research, we also identified a similar polypeptide at 56.18 kDa in the CSL extract, aligning with the findings of Innocenti et al*.* [11].

Another study by Tabouret et al*.* [12] highlighted specific protein bands in different extracts of *O. ovis* larvae, particularly a 28.0 kDa protein that was highly reactive in immune tests. Our study found polypeptides at 38.66 kDa and 28.91 kDa in the CSL extract, which matches the findings of Tabouret et al. [12].

Similarly, Arunkumar et al*.* [13] identified eight specific protein bands in the excretory-secretory antigens of *Oestrus ovis* larvae, with distinct molecular weights. Interestingly, our study detected polypeptides at 63.63 kDa, 56.18 kDa, 40.30 kDa, 31.42 kDa, 23.49 kDa, and 16.17 kDa, aligning closely with the findings of Arunkumar et al. [13]. This consistency validates the presence of these specific polypeptides in the excretory-secretory antigens of *Oestrus ovis* across.

5. CONCLUSION

The analysis of the crude somatic pooled L_2 and L_3 antigen (CSL) from *Oestrus ovis* using SDS-PAGE showcased a diverse array of 27 polypeptides with molecular weights ranging from 14.27 kDa to 81.64 kDa. This diversity in molecular sizes underscores the complex nature of the CSL protein, highlighting the various protein components present within the larval stages.

Understanding the molecular composition of the CSL antigen serves as a foundational step towards further investigations into the immunogenicity and potential applications of these proteins in the diagnosis, treatment, or management of nasal myiasis caused by *Oestrus ovis*. This comprehensive analysis lays the groundwork for future studies focusing on specific polypeptides and their potential roles in immunoreactivity or therapeutic development.

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.

ACKNOWLEDGEMENTS

The research facilities provided by P. V. Narasimha Rao Telangana Veterinary University Rajendranagar, Hyderabad is being acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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