The Antigenic Structure Characterization of *Oestrus Ovis* Larvae

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Abstract
With the aim of proteic components definition from *Oestrus ovis* larvae, endowed with antigenic properties, able to induce immune responses *in vivo* and to react *in vitro* with induced molecular effectors were been performed: electrophoresis in poliacrilamid gel, western blot technique preceded by immunotransfer, immunoassay test. Total soluble larval antigens of *O. ovis* were been prepared through ultrasonic disintegration, from all three larval stages. Western blot technique allowed and emphasized the specific antigens with a superior sensitivity in comparison with SDS-PAGE electrophoresis. After antigenic characteristics demonstration of investigated larval antigens were been performed the immunoassay test to emphasized the antibodies dozes for *O. ovis* infestation diagnosis.

Key words: antigens, electrophoresis, immunotransfer, oestrus ovis larvae, western blot

1. Introduction

Ovine oestrosis is one of the parasitic disease, with an evolution influenced by a great number of biotic and abiotic environmental factors. Because of environmental factors influence, the disease evolves with a different intensity from a region to another, from a year to another, thus economic and sanitary-veterinary effects are ignored in many situations or assigned to another diseases that ovine oestrosis can be associated. Taking into consideration that the researches from our country aimed in a small measure immunologic aspects of *O. ovis* parasited animals, performed research was been searched the parasited animals immunological status defining and a larval antigenic structure characterization with the object of precocious immunologic diagnosis methods identification [1].

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2. Materials and methods

The antigenity studies in protein fractions from *O. ovis* larvae structure were been performed on blood samples from 10 Merinos of Transilvania breed sheep 2-5 years aged with clinical signs of oestrosis manifested through: sneeze, bilateral seromucouse and mucopurulent nasal drains, head shaking. Parallel were been taken blood samples from 10 same aged and breed clinically healthy sheep provided by a particular farm, treated against ectoparasites twice per annum, in spring and in autumn. In the aim of *O. ovis* larvae protein compounds definition, endowed with antigenic properties capable to determine in vivo immune responses and to react in vitro with induced molecular effectors [2], were been performed: electrophoresis in poliacrilamid gel, western blot technique preceded by immunotransfer technique and immunoassay test. The marked out bands after enzymatic reaction, specified mode coloured were been considered specific antigenic fractions. In this way was been established that in a total antigen composition are present, in a certain number, specific antigens, capable to induce

adapted immune responses and to interact with sinthetized specific immunoglobulins [3].Total soluble larval antigens of *O. ovis* were been prepared trough ultrasonic disintegration. *Oestrus ovis* larvae were been minced in 20 ml PBS. The obtained solution was ultrasonicated 30 minutes at 4°C (in ice bath); the antigen represented by big larvae was diluted with another 20 ml PBS and again ultrasonicated 30 minutes at 4°C (the obtained suspension was very viscid). The obtained suspension was 30 minutes centrifugated at 10 000 rotations/minute. The antigen prepared from L_2 and L_3 larvae (second and third developing stage Oestrus ovis larvae) had a total protein content of 2.15 mg/ml (Ag I). The antigen prepared from L_1 larvae (first developing stage Oestrus ovis larvae) had a total protein contain of 0.52 mg/ml (Ag 2).

3. Results and discussion

Four samples of *O. ovis* larvae disintegrated were been obtained through ultrasonication in PBS, at 4°C in an adequate regime (samples 1 and 3) and through ultrasonication and boiling one hour (samples 2 and 4).

After cold centrifugation the aqueous supernatants representing total soluble larval antigens were been separated. The four larval antigens were been characterized: biochemical, through total protein contain establishing (Biuret method); electrophoretic, through SDS-PAGE; antigenic, through Western blot technique, preceded by immunotransfer. The results of total protein content determination are shown in Table 1.

Table 1. Biochemical characterization of larval antigens

Sample number	*O. ovis* larvae (dimension)	Processing through	Total proteins (mg/ml)
1	big (L_2, L_3)	U.S.	2.15
2	big (L_2, L_3)	U.S.	1.32
3	small (L_1)	U.S. + warm treatment	0.52
4	small (L_1)	U.S.	0.29

It was been observed that the two total antigens prepared from IInd and IIIrd stage of larval development had a protein (2.15 and 1.32 mg/ml) superior contain in comparison with antigen obtained from Ist larval stage (under 1 mg/ml). This will influence the subsequent tests results which will give decisive results, through electrophoresis, only in the case of antigens more abundant in total proteins. In first sample of antigen (2, 3 bands) constituted by ultrasonicated IInd and IIIrd larval stages, the obtained fractions are clear and well contoured. In the other samples the electrophoretic fractions were not clear identified and adequate characterized. In table 2 are presented in synthesis the molecular weight of protein fractions contained in total soluble antigens emphasized through electrophoresis (electrophoretic fractions) and Western blot (antigenic fractions).

Table 2. Results of electrophoretic and immunotransfer (Western blot) study

Electrophoretic fractions	Antigenic fractions	
Sample 1 (double) L_1, L_3 (kDa)	Sample 1 (double) L_2, L_3 (kDa)	Sample 3 (double) L_1 (kDa)
79.43	77.19	-
66.08	64.78	-
60.00	58.77	-
46.76	43.03	38.28
33.12	34.05	34.05
27.54	28.02	-

Analyzing the obtained results can be observed that all protein fractions from total soluble larval antigens are distributed in 27-80 kDa domain. The same fractions (with small formula weights differences, owed to changes occurred in the time of supplementary treatments in immunotranfer and Western blot) proved to be antigenic in vitro (through coupling reaction with specific antigens).The studied larval antigens can be use like reagents in antigen-antibody reactions in the aim of antibodies emphasizing and dosage in *O. ovis* infestation diagnosis [4,5]. Data obtained through immunoassay test (ELISA) emphasized that the obtained antigens both from Ist and IInd,

III[rd] stage of development larvae have specificity towards seric antibodies synthesized in natural infestation with O. ovis larvae. In immunoassay test were been used more purified antigens obtained from I[st] (L1) and II[nd] (L_2) and III[rd] (L_3) larval stages. Antigen Ag 1 prepared from L_2 and L_3 had a total protein concentration of 2.15 mg/ml and Ag 2 antigen prepared from L_1 had a total protein concentrationof 0.52 mg/ml. Table 3 shows the results obtained through Ag 1 using marked out that most high values were been obtained when in reaction was used concentration of 10 µg/ml sample, and serums had 1:100 dilution. Thus, media of positive/negative serum report in the case of antigen utilization of 10 µg/ml concentration was bigger at serum dilution of 1/100 than 1/50 dilution.

Table 3. Antibodies titre on dilutions of Ag 1

No. dilution		5 µg/ml (D.O.)	P/N	10µ (D.O.)	P/N	20 µg/ml (D.O.)	P/N	40 µg/ml (D.O.)	P/N
1.	1:50	1326	9.1	1600	10.8	1700	10.0	2100	10.8
2. Ser	1:100	1135	12.1	1233	11.5	1393	11.0	1657	11.0
3.	1:200	790	-	850	-	938	-	1118	-
4.	1:50	837	5.7	925	6.25	1054	6.2	1508	7.7
5. Ser 4	1:100	644	6.9	692	6.5	959	7.6	1201	7.9
6.	1:200	457	-	538	-	731	-	937	-
7.	1:50	913	6.2	998	6.7	1291	7.8	1627	8.3
8. Ser 5	1:100	751	8.0	828	7.7	1090	8.65	1371	9.1
9.	1:200	555	-	628	-	819	-	1002	-
	1:50	1025	6.9	1174.3	7.9	1384.3	7.9	1745	8.9
Media	1:100	843.3	8.9	917	8.6	1147.3	9.1	1409.6	9.3
	1:200	600.6	-	672	-	829.3	-	1019	-
10. N	1:50	147	-	148	-	170	-	195	-
11.	1:100	94	-	107	-	126	-	151	-

N=negative serum, P/N=positive/negative serum report, D.O.=optic density

Also, was observed that through bigger antigen concentrations utilization (20 µg/ml and 40 µg/ml) the report between antibodies values titre (positive serum and negative serum) not preserved the proportion with utilized antigen concentration.

In the case of antigen Ag 2 utilization the obtained results are similar, with the mention that the report between antibodies concentrations from researched serums with those registered in control samples were more reduced. The highest values of positive serum/negative serum were obtained when in reaction the serums were applied in 1/100 dilution (Table 4).

Table 4. Antibodies titre on dilution of Ag 2

No. dilution		5 µg/ml (D.O.)	P/N	10µ (D.O.)	P/N	20 µg/ml (D.O.)	P/N	40 µg/ml (D.O.)	P/N
1.	1:50	872	4.2	952	3.8	1078	4.2	1302	4.6
2. Ser	1:100	686	4.6	746	5.1	878	4.6	1084	5.3
3.	1:200	435	-	501	-	593	-	710	-
4.	1:50	830	4.9	936	3.8	1125	4.4	1382	4.9
5. Ser 4	1:100	630	4.3	731	5.0	929	4.9	1213	5.9
6.	1:200	443	-	529	-	713	5.2	908	-
7.	1:50	860	4.5	1104	4.4	1319	5.4	1552	5.5
8. Ser 5	1:100	733	4.9	863	5.9	1038	-	1324	6.4
9.	1:200	517	-	635	-	678	-	957	-
	1:50	854	4.1	997.3	4.0	1174	4.6	1412	4.9
Media	1:100	683	4.6	780	5.3	948.3	4.9	1207	5.8
	1:200	465	-	555	-	661.3	-	858.3	-
10. N	1:50	211	-	249	-	255	-	283	-
11.	1:100	148	-	146	-	191	-	206	-

N=negative serum, P/N=positive/negative serum report, D.O.=optic density

Analyzing the obtained results regarding to optimal concentration was observed that is 10 µg/sample, the value of P/N report is 5.3. The application of antigen bigger concentrations is not managing to significant increasing of this report and it is not justified. The researches in the view of evaluation of immune specific response through antibodies synthesis were made on five serums provided from naturally infested animals with the two studied antigens. The obtained results emphasized that highest average values were in the case when in reaction was applied the antigen prepared from *O. ovis* larvae in II and III stage of development (Ag 1) with a total protein concentration of 2.15 mg/ml (Table 5).

Table 5. Specific immune response evaluation

Specificatioon	Ag 1 (D.O.)	P/N	Ag 2 (D.O.)	P/N
Serum				
1	1090	10.9	527	5.2
2	978	9.8	336	3.4
3	1118	11.2	951	9.5
4	921	9.2	749	7.49
5	932	9.32	747	7.47
X±Sx	10007.8±81.3	-	662 ±211.1	-
C.V.(%)	8.07	-	31.89	-
N	100	-	100	-

Comparing the obtained results regarding antibodies titre in function of utilized antigen, can be assert that the most increased values were been obtained in the case of antigen prepared from L_2 and L_3 *O. ovis* larvae, respectively average value 1007.8 D.O. and C.V. 8.07%. In the case of antigen prepared from L_1, antibodies titre was 662.0 and C.V. 31.89%. The obtained results regarding adaptative immunological reactivity in sheep naturally infested with *O. ovis* larvae emphasized a reaction manifested through specific antibodies synthesis, with mention that intensity of reaction depends on larvae evolutionary stage. The reaction is more intense when *O. ovis* larvae reached the Ist and IIIrd stage of evolution, demonstrated in the research obtained data.

4. Conclusions

Soluble larval antigens of O. ovis were been prepared through ultrasonic disintegration. The two investigated samples represented antigens from IInd, IIInd and antigen from Ist stage of larval development. The obtained antigens were been characterized through electrophoresis in poliacrilamid gel (SDS-PAGE technique) and through Western blot technique, preceded by fractions transfer from the total antigens structure.
In prepared antigen from IInd and IIIrd larval stage of development were been emphasized six antigenic fractions with molecular weights situated in 27–80 kDa domain, with two fractions clear and intense expressed. The same fractions were been observed through SDS-PAGE electrophoresis too. In antigen obtained from Ist stage of larval development was been noted the presence of two antigenic fractions powerless expressed, with molecular weights in 34-38 kDa domain, unobserved in SDS-PAGE electrophoresis.
Western blot technique allowed and dignified the specific antigens with a higher sensitivity in comparison with SDS-PAGE electrophoresis, due to an efficient detection system using, represented by antigen-antibody and enzymatic reactions.
The optimal concentration of reaction applied antigen was 10 µg/ml sample, and 1:10 serums dilution. The antigen obtained from stages II and III of *O. ovis* larval development had a more pronounced immunogenity than those obtained from stage I.
The immunoassay test may offer sufficient exactly data regarding antibodies titre with the condition of more purified antigen obtaining.

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