ISOLATION, PURIFICATION AND IMMUNO-BIOCHEMICAL CHARACTERIZATION OF IMMUNOGLOBULIN OF JAMUNAPARI GOAT (*Capra hircus*)

N. N. FARUQUEE, S. BATABYAL, S. CHATTOPADHYAY, A. MAITY*, P. K. DAS
K. BATABYAL, S. S. KESH, R. TUDU AND A. BARUI

*Faculty of Veterinary and Animal Sciences
West Bengal University of Animal and Fishery Sciences
Kolkata- 700 037, India*

Jamunapari is one of the largest and most popular goat breeds in India. The present study was carried out to purify and characterize the IgG of Jamunapari goat since IgG plays central role in the fight against infectious diseases. The IgG of Jamunapari goat could be isolated by salting out (50% saturated ammonium sulfate solution) and purified by the gel filtration chromatography on Sephacryl S-200. The Jamunapari goat immunoglobulin G (IgG) was obtained in relatively purified form. The purified Jamunapari goat IgG revealed major bands of 61 kDa, 55 kDa, suggestive of heavy chains and 28kDa, 23 kDa, suggestive of light chains. The purified immunoglobulin has been found to be immunoreactive by DID and western blot analysis in the present study.

Key words: DID, IgG, SDS-PAGE, Western blot

IgG is the predominant immunoglobulin in the blood and primarily involved in secondary immune response in animals (Talwar and Gupta, 1992). It is the only antibody that can cross placenta. In the serum, IgG is the antibody class with the highest concentration, approximately 0.5-3 gm/dL, with some species differences (Tizard, 2009). IgG₁ and IgG₂ are the two IgG subclasses universally distinguished

(Murphy *et al.*, 1964; Aalund *et al.*, 1971) which differ in their physico-chemical and biological properties. IgG₁ is the main component of milk and saliva secretion, and IgG₂ is found in blood serum, being main serum immunoglobulin. IgG₁ has a molecular mass of 163KDa and IgG₂ of 150KDa (Wernicki, 1988). Based on immunoelectrophoretic aspects and column separation with DEAE-Sephadex, the

*Corresponding Author

presence of a third IgG subclass has been hypothesized (Jeffrey, 2007). A fourth class of immunoglobulins having similar properties to the human IgE has been identified in bovine (Wells and Eyre, 1972), ovine (Hogarth-Scott, 1969) and caprine species.

Jamunapari is one of the largest goat breeds in India; it has been extensively utilized to upgrade indigenous breeds for meat and milk, and has been taken to neighboring countries for the same purpose. This breed of goat needs proper attention and conservation. It is only possible under strict disease monitoring system for which purified IgG may be necessary for enzyme immunoassays. Purification of IgG is simple due to its charge properties, high molecular weight and abundance in normal serum. There is dearth of available literature on the IgG status of Jamunapari goats. Therefore, this study was carried out to isolate and purify the IgG by gel filtration chromatography from Jamunapari goat serum and to characterize the purified IgG immuno-biochemically by SDS-PAGE and western blot.

MATERIALS AND METHODS

The Blood sample of Jamunapari Goat was collected from three different apparently healthy animals maintained in organized farm around Kolkata and serum was separated.

Precipitation of immunoglobulins was carried out in 50% saturation, by adding

3.13 gm of ammonium sulphate to 10 mL of serum sample. The salt was added in batches slowly with efficient stirring at 20 - 25°C. After 2 hours serum was centrifuged at 10,000 rpm for 15 minutes. The pellet obtained was dissolved in PBS (pH-7.2) of original serum volume and 50% ammonium sulphate precipitation was repeated. Finally the serum was centrifuged after 2 hours as above and the pellet was dissolved in PBS.

The ammonium sulphate precipitated immunoglobulin was taken in a dialysis bag and dialyzed with several changes of PBS (pH- 7.2) for 24 hours at 4°C. This was done to remove the excess ammonium sulphate. Thus crude IgG was obtained (Green and Hughes, 1955).

Protein concentration was estimated for the dialyzed crude IgG of Jamunapari goat by Lowry's method (Lowry *et al.*, 1951). The absorbance was measured at 750 nm by UV-VIS Spectrophotometer (Systronics-119).

Purification of IgG by gel filtration chromatography: 1.5 mL of purified Jamunapari goat immunoglobulin (crude IgG) was applied to a column of Sephacryl S-200 HR (2 cm diameter and 42 cm in length), with a bed volume of 132 mL. Elution was carried out by a buffer containing PBS (pH- 7.2), PMSF (0.03mM) and 0.02% sodium azide at a flow rate of 35 mL per hour. Fractions of 3.5 mL each were collected in 36 different sterile test tubes. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV-VIS spectrophotometer (Systronics-119).

Isolation, characterization of IgG of Jaminapari goat

Fig. 1. 10% SDS-PAGE showing the purified IgG fraction (S) along with protein molecular weight marker (M)

Fig. 2. Double immunodiffusion showing the precipitin line of IgG with the hyperimmune serum (A: Hyperimmune serum; B: Jamunapari goat serum IgG; C: Normal control serum)

Fig. 3. Western bolt of purified IgG from Jamunapari goat serum

Graph 1. Purification of crude IgG of Jamunapari goat serum by gel filtration on Sephacryl S-200

A graph was plotted by taking fraction no. along the X axis and absorbance in Y axis, which revealed a bell shaped curve with a major peak. Then the fractions of the peak were pooled.

Protein concentration for the pooled fractions was estimated by Lowry's method (Lowry *et al.*, 1951). Then the fractions were concentrated by dialysis against sucrose using dialysis membrane. The concentrated peak fractions were preserved at -20°C for further use.

SDS-PAGE of Jamunapari goat IgG:

The crude and purified samples were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-

PAGE) in vertical slab gel electrophoresis instrument (AE-8450) with power pack (ATTO Corporation, Japan) under identical experimental condition according to the method described by Laemmli (1970). Molecular weights were determined after SDS-PAGE using protein molecular weight markers (PMW-M, Bangalore Genei).

Raising of antiserum: The antibody against crude Jamunapari goat (*Capra hircus*) serum was raised in healthy New Zealand white rabbit with an average weight of 1.5 Kg. The crude antigen was thoroughly mixed with equal volume of Freund's complete adjuvant (FCA) (1:1) and a total of 1 mL was injected intramuscularly at 2 sites into the thigh

muscle. Four booster doses of the same antigen emulsified with Freund's incomplete adjuvant (FIA) (1:1) were given with subsequent increase in amount after 7 days interval following the first injection. Blood was collected from the rabbit 7 days after the last booster dose and serum was separated by centrifugation at 5000 rpm for 10 min. The antiserum was stored at -20°C for further use.

Double immunodiffusion test (DID): The DID test was performed according to Hudson and Hay (1989) with some modifications.

Western blotting: Immunochemical analysis of purified protein was done by western blot technique according to Towbin *et al.* (1979) with some modifications. The proteins were separated by 15% SDS-PAGE (Laemmli, 1970) and then the resultant proteins were electroblotted to nitrocellulose filter paper (Immobilon- NC).

RESULTS

Crude IgG was obtained after 50% ammonium sulphate precipitation of Jamunapari goat serum and subsequently it was dialyzed against PBS. The mean protein concentration of the crude IgG from Jamunapari goat serum was 24.06 ± 1.97 mg/mL. Purified Jamunapari goat IgG was prepared by gel filtration chromatography on Sephacryl S-200 in which the proteins were resolved into one major peak. The fractions showing the peak (Graph1) were

pooled together. The mean protein concentration of the pooled fraction was 8.68 ± 1.22 mg/mL. The fractions were concentrated by dialysis using sucrose.

10% SDS-PAGE of the pooled fraction revealed major protein bands of 61 kDa, 55 kDa, 28 kDa and 23 kDa (Fig. 1).

Single precipitin line was observed in DID test when the purified immunoglobulin was reacted with the hyper-immune serum raised in rabbit. No precipitin line or band was found against the normal control serum (Fig. 2).

The purified immunoglobulin was found to be immunoreactive against hyperimmune serum raised in rabbit analyzed by western blot technique (Fig. 3).

DISCUSSION

In the present study, SDS PAGE of purified IgG obtained by Gel filtration chromatography revealed bands of 61 kDa, 55 kDa and 28 kDa, 23 kDa from Jamunapari goat serum resembling heavy chains and light chains respectively.

The present study corresponds almost to the results of Ninfali *et al.* (1994) who obtained two heavy chains of 50 kDa and two light chains of 25 kDa of goat immunoglobulin purification on phosphocellulose and DEAE affi-gel blue chromatography followed by 7.5 % SDS-PAGE.

Boden *et al.* (1995) obtained bands of 55 kDa and 25 kDa for heavy and light chains

after purification of goat immunoglobulin by immobilized metal ion affinity chromatography.

Purified buffalo IgG₁ and IgG₂ were found to be having heavier 58 kDa chains, identified as H chains; and lighter 24 kDa chains, identified as L chains; observed by Kakker and Goel (1993) which is in agreement with our study.

Double immunodiffusion test showed single precipitin line when purified Jamunapari goat IgG reacted against hyperimmune serum raised in rabbit. This corresponds to the bovine IgG₂ infraclass purification that showed single precipitin line (Krishnamohan and Giridhar, 1991). Ramesh and Krishnamohan (1992) observed a single precipitin line with DEAE cellulose purified ovine IgG.

The purified immunoglobulin was found to be immunoreactive against hyperimmune serum raised in rabbit when analyzed by western blot. Both the heavy and light chain

of purified immunoglobulin G of Jamunapari goat showed immunoreactivity in the NCP.

Rantamaki and Muller (1995) found the goat IgG to be immunoreactive when immune blotting was done with an antiserum against the human $\alpha 2$ -macroglobulin and subsequently with the PG-peroxidase conjugate.

It can be concluded that, the IgG of Jamunapari goat could be isolated by salting out (50% saturated ammonium sulfate solution) and purified by the gel filtration chromatography on Sephacryl S-200. The Jamunapari goat immunoglobulin G (IgG) was obtained in relatively purified form. In this present study the purified Jamunapari goat IgG revealed major bands of 61 kDa, 55 kDa, suggestive of heavy chains and 28kDa, 23 kDa, suggestive of light chains. The purified immunoglobulin has been found to be immunoreactive by DID and western blot analysis in the present study.

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