PART I: GENERAL INFORMATION

1. Name of the Institute/University/Organisation submitting the Project Proposal:

   Indian Veterinary Research Institute, Izatnagar, Bareilly U.P, India

2. State: Uttar Pradesh

   Status of the Institute: Deemed University (Indian Council of Agricultural Research) (ICAR), New Delhi

(Please see Annexure-I)

4. Name and designation of the Executive Authority of the Institute/University forwarding the application:

   Prof. M.C Sharma,
   Director,
   Indian Veterinary Research Institute. Izatnagar- Bareilly, U.P.

5. Project Title: Silencing of protease genes in Fasciola gigantica eggs by RNA interference for developing parasite transmission blocking strategy

6. Category of the Project (Please tick): R&D / Programme Support

   Research and Development

7. Specific Area (Please see Annexure - II): RNA interference

8. Duration: 3 Years

9. Total Cost (Rs.) 45.6 lakhs

10. Is the project Single Institutional or Multiple-Institutional (S/M): Single Institutional

11. If the project is multi-institutional, please furnish the following:

   Name of Project Coordinator: NA
Fasciolosis caused by the helminth parasite *Fasciola gigantica* and *F. hepatica*, impedes the livestock production due to the adverse effects of the parasite on the health and productivity of the animal. Efforts on the development of a viable vaccine have been hampered by the strong immune evasion mechanisms of the parasite. Therefore, alternative strategies for controlling the parasite infection are needed to be pursued. The proposed research project envisages identification of the proteins involved in the hatching process of the eggs in the helminth parasite. As no information is available on the mechanisms of hatching process of the parasite egg, understanding the role of specific proteins in the release of larvae from the eggs will form the basis for developing intervention protocols for blocking the transmission of the parasite. This study will be a crucial step in the development of transmission blocking strategy and saving the environmental contamination by the parasite. The proposed research project aims at identifying the proteases involved in the larval hatching from the parasite eggs using RNA interference. This study will form a basis for exploiting these molecules in blocking the parasite eggs from hatching in the environment, thereby disrupting the parasite life cycle. Also, the proteases once characterized functionally in the egg stage of the parasite can be exploited in its other developmental stages for understanding their role in various metabolic processes of the parasite.

13. **Project Summary** (Not to exceed one page. Please use separate sheet).

Parasitic diseases are a major problem world wide, being not only a health issue but when affecting productive animals, an important factor in the economy. *Fasciola* (liver fluke) is a parasitic helminth of major socio-economic importance in both tropical and temperate countries and fasciolosis, caused by *F. hepatica* and *F. gigantica*, is a leading cause of production losses to the livestock and meat industries due to clinical disease, reduced weight gain, reduced milk production and deaths. Vaccination is a viable strategy for controlling the disease caused by *Fasciola* but no vaccine could be developed so far. Strong immune evasion mechanisms developed by the adult parasite have hampered vaccine development against this scourge. Alternate control strategies include development of transmission blocking methodologies by focusing on identifying the target molecules relevant to hatching of the parasite eggs. These molecules can be the future targets for intervention approaches for blocking the transmission of the parasite in the natural environment. But no research work has been conducted on the identification of proteins responsible for hatching of the parasite egg.

Two important proteases of *Fasciola gigantica* namely cysteine and serine proteases were found essential for the hatching of the larva (miracidium) from the eggs *in vitro* in a study in our laboratory, using serine and cysteine protease inhibitors. This study has highlighted a crucial role for these two enzymes in the egg hatching process for *F. gigantica*. The role of these two proteases in the hatching process of the *Fasciola* eggs as determined in this work needs to be confirmed at the molecular level by knock down studies of genes coding for these proteins. Therefore, the present research project is proposed to target *Fasciola gigantica* genes coding for cysteine and serine proteases, along with other relevant protease leucine aminopeptidase in the parasite egg stage.
These genes will be knocked down using RNA interference and the silencing effect of these genes on the hatching of the larvae from the eggs will be evaluated using standard RNAi protocols. Knock down of the target proteases by RNAi will be evaluated at the transcriptional level by quantitative real-time PCR and at the translational level by western blotting and confocal microscopy. These experiments will precisely identify the role of these proteases in the hatching process of the eggs in this pathogen. Once the role of these proteases in egg hatching process is established, these molecules will be the crucial targets in the future development of a transmission blocking strategy and saving the environmental contamination by the parasite. These proteases will also constitute new targets for their future exploitation in the drug and vaccine development.

PART II: PARTICULARS OF INVESTIGATORS
(One or more co-investigators are preferred in every project. Inclusion of co-investigator(s) is mandatory for investigators retiring before completion of the project)

Principal Investigator:

14. Name: **Dr. O.K. Raina**

   Date of Birth: 20th April 1958   Sex (M/F): Male

   Designation: Senior Scientist

   Department: Division of Parasitology,
   Institute/University: Indian Veterinary Research Institute

   Address: IVRI, Izatnagar- Bareilly, U.P., India

   PIN: 243122
   Telephone: 09458269041   Fax: 0581-2302368.

   E-mail: rainaok@rediffmail.com

   Number of research projects being handled at present: Nil

Co-Investigator-I

15. Name: **Dr. Praveen Gupta**

   Date of Birth: 27th August 1965   Sex (M/F): Male

   Designation: Senior Scientist

   Department: Division of Veterinary Biotechnology

   Institute/University: Indian Veterinary Research Institute

   Address: IVRI, Izatnagar, Bareilly, U.P., India

   Telephone: 09456408509   Fax: 0581-2302368
Co-Investigator-II

16. Name: Dr. P. Joshi

Date of Birth: 20th August 1956   Sex (M/F): Male
Designation: Principal Scientist.
Department: Division of Biochemistry,
Institute/University: Indian Veterinary Research Institute
Address: IVRI, Izatnagar, Bareilly, U.P., India.
PIN: 243122
Telephone: 09897279493   Fax 0581-2302368
E-mail: pj@ivri.res.in

Number of Research projects being handled at present: Two

Note: Use separate page, if more investigators are involved
16. **Introduction** (not to exceed 2 pages or 1000 words)

Parasitic diseases are a major problem world wide, affecting productive animals that constitute an important factor in the livestock economy. *Fasciola*, also called a liver fluke, is a parasitic helminth of major socio-economic importance in both tropical and temperate countries and fasciolosis, caused by *F. hepatica* and *F. gigantica*, is a leading cause of production losses to the livestock and meat industries due to clinical disease, reduced weight gain, reduced milk production and deaths. Tropical fasciolosis is an impeding factor in the growth and productivity of domestic animals including sheep, cattle, buffalo and goats, leading to a considerable loss to the livestock economy, with a conservative estimate of US $3 billion loss per annum worldwide (Spithill *et al.*, 1999). Development of control measures for fasciolosis is a national priority for improving the production and quality of livestock products. Fasciolosis is an emerging zoonosis throughout parts of Middle East, Asia and South America, adding considerably to the human sufferings.

Vaccination is a viable strategy for controlling the disease caused by *Fasciola* but no viable vaccine could be developed so far. Limitations to the vaccine development are the complex mechanisms of immune evasion developed by the parasite and lack of understanding of the different components of the natural rejection processes including recognition of target antigens, induction of protective immune response phenotypes and activation of the appropriate immune effector mechanisms. Chemotherapy is the only control measure and no new drug has been approved for the last three decades. Currently there is a significant focus on the development of new approaches for the prevention and control of fasciolosis in livestock. The expanding genomic and expressed sequence tag (EST) datasets for parasitic flat worms including *Fasciola* have helped highlight the need for technologies to aid their exploitation. Until recently, parasitic flat worms have proven refractory to genetic manipulation (Morales *et al.*, 2007) and absence of a tractable and appropriate model species, such as that provided for nematodes by *Caenorhabditis elegans*, has repressed scientific progress associated with the elucidation of gene function. Exciting advances in functional genomics for parasitic helminths are starting to occur, with transgene expression and RNA interference (RNAi) reported in several species of nematodes but the area is still unexploited for determining the virulence factors, drug and vaccine targets in *Fasciola*, where research has been hampered in the absence of functional genomics studies. The existence of a viable and functional RNAi pathway in *Fasciola hepatica* was discovered recently (Mcgonigle *et al.*, 2008; Rinaldi *et al.*, 2008) that has established the starting point for functional genomics studies in *Fasciola*. Functional genomics study through RNAi on *Fasciola gigantica* (tropical liver fluke) will offer new opportunities for the identification of key mediators in the parasite-host interactions that could be exploited as future drug and vaccine targets. Therefore, the breakthrough achieved recently (Mcgonigle *et al.*, 2008) in determining the existence of a functional RNAi pathway in *F. hepatica* needs to be exploited in this tropical fluke, by initiation of research in this field of functional genomics, to achieve the ultimate goal of development of a vaccine and therapeutic intervention.

In view of the strong immune evasion mechanisms developed by the adult parasite that has hampered vaccine development against this scourge, alternate control
Strategies for this parasite include development of transmission blocking methodologies. Therefore, research needs to be focused on identifying the target molecules relevant to blocking the transmission of the parasite in its natural environment. No research work has been conducted on identification of proteins responsible for the hatching of the parasite larvae during the life cycle of the parasite. In a related parasite Schistosoma mansoni leucine aminopeptidase (LAP) plays a central role in the hatching of the miracidium from the egg (Xu et al., 1990) that was confirmed by RNAi studies (Rinaldi et al., 2009). However, expression of leucine aminopeptidases in the eggs of F. gigantica has not been determined. Fasciola parasites produce both cathepsin B and L proteases (Cancela et al., 2008; Grams et al., 2001; Meemon et al., 2004; Wilson et al., 1998) and direct evidence for the importance of cysteine proteases in liver fluke biology was provided by a recent study on silencing of F. hepatica CatB-1 and CatL-1 by RNA interference in newly excysted juveniles, which revealed a significant reduction in the gut penetration of this parasite stage in vitro, following knock down of the expression of each enzyme (McGonigle et al., 2008). But there is no direct evidence on the role of cysteine and serine proteases or leucine aminopeptidases or for that matter role of other proteins in the egg hatching of Fasciola parasite; though in a study in our laboratory, use of serine and cysteine protease inhibitors caused a complete inhibition of the F. gigantica egg hatching, thereby highlighting a crucial role for these two enzymes in the egg hatching process for F. gigantica.

The role of cysteine and serine proteases as well as leucine aminopeptidase in the hatching process of the Fasciola eggs as determined in our previous work needs to be confirmed using RNAi. Therefore, the present research project is proposed to target the gene signals of these molecules by RNAi to precisely identify the proteins involved in the hatching of this parasite eggs. This study will be a crucial step in the future development of a transmission blocking protocols and saving the environmental contamination by the parasite and will also lead to the identification of new targets for their future exploitation in the drug and vaccine development.

16.1 Origin of the proposal

There is no scientific information available on the role of different proteins in the egg hatching process of Fasciola. Knowledge on this aspect is very crucial for the development of future transmission blocking strategies for the parasite. No viable vaccine could be developed against fasciolosis. Therefore, developing a strategy on blocking the transmission of the parasite in the host populations is a viable alternative. Present research proposal is envisaged for identification of the target molecules in the egg stage of the parasite that are responsible for the hatching process of the larvae from the eggs, using functional genomics approach of RNAi. Studies on the inhibition of cysteine and serine proteases and leucine aminopeptidases in the eggs of F. gigantica in our laboratory with protease specific inhibitors caused complete inhibition of the egg hatching. These studies highlight the role of these proteases in the egg hatching. But these results need to be confirmed at the molecular level by knocking down the genes coding for these proteases for precisely pinpointing the role of these proteins in the egg hatching process. This study will ultimately lead to the development of alternative strategy of transmission blocking for the parasite and also will help in identifying novel targets in the parasite for their future exploitation in the drug and vaccine development.
Currently there is a significant focus on the development of new approaches for the prevention and control of fasciolosis in livestock. The expanding genomic and EST datasets for *Fasciola* have helped highlight the need for technologies to aid their exploitation. Until recently, parasitic flat worms (trematodes) have proven refractory to genetic manipulation (Morales et al., 2007) that has repressed scientific progress associated with the elucidation of gene function. Advances in functional genomics for parasitic helminths are starting to occur, with transgene expression and RNAi reported in several species of nematodes but the area is still unexploited for determining the virulence factors, drug and vaccine targets in *Fasciola*, where the research has been hampered in the absence of functional genomics studies. The existence of a viable and functional RNAi pathway in *F. hepatica* discovered recently (Mcgonigle et al., 2008; Rinaldi et al., 2008) has established the starting point for functional genomics studies in this parasite. Functional genomics study through RNAi on the tropical liver fluke *F. gigantica* will, therefore, offer new opportunities for the identification of key mediators in the parasite-host interactions that could be exploited as future drug and vaccine targets. Hence, the recent breakthrough in determining the existence of a functional RNAi pathway in *Fasciola* needs to be exploited in this tropical liver fluke by initiation of research in this field, to achieve the ultimate goal of development of a vaccine or blocking transmission of the parasite or therapeutic intervention against fasciolosis.

**Hypothesis**

We hypothesize for a role of two *F. gigantica* proteases namely cysteine and serine protease in the hatching process of the larvae from the eggs. Leucine aminopeptidase’s role in the egg hatching also needs to be elucidated. This hypothesis will be confirmed by undertaking studies on knock down of genes coding for these proteins in the egg stage of the parasite and evaluating the role of these proteins in the hatching process.

**Key Questions**

Role of cysteine and serine proteases and leucine aminopeptidase of *F. gigantica* in the hatching process of the eggs needs to be evaluated.
The hatching of eggs in different helminth parasites has recently been established. In Schistosoma mansonii, RNAi has been exploited for functional studies of different genes. However, no such studies have been done in Fasciola, except for the above two reports of Rinaldi et al. (2008) and Mcgonigle et al. (2008) on the gene silencing of proteins involved in the gut penetration. In India, work on the reverse genetics including RNAi, transgenesis and knock out studies have not been initiated on helminths of veterinary importance. Lack of such studies has hampered identification of virulence factors, drug and vaccine targets in this parasite.

16.6 The relevance and expected outcome of the proposed study

The proposed project will establish the role of various proteases in the egg hatching process in F. gigantica. This information will be utilized in the future strategies on blocking the transmission of the parasite between the host populations. At present no viable vaccine has been developed against this scourge due to strong parasite immune evasion mechanisms and lack of understanding of the different effector mechanisms of the host immunity. In such a situation the strategy of hitting the parasite at the egg stage by preventing its hatching process is a viable alternative to control the spread of the parasite.

16.7 Preliminary work done so far

No scientific data is available on the mechanism of hatching process of the eggs in Fasciola parasite. Role of cysteine / serine proteases and leucine aminopeptidases or for that matter role of other proteins in the egg hatching of Fasciola is not known. A study in our laboratory was conducted for elucidating the role of leucine aminopeptidases and serine and cysteine proteases in F. gigantica egg hatching process using protease specific inhibitors. Cysteine and serine protease inhibitors caused a complete inhibition of the F. gigantica egg hatching, thereby highlighting a crucial role for these two enzymes in the egg hatching of F. gigantica.

Therefore, the role of these proteases in the hatching process of the Fasciola eggs as determined in our previous work needs to be confirmed using RNAi, where the genes for these proteases will be the targets for RNAi. This will be an important step in the future development of a transmission blocking strategy and saving the environmental contamination by the parasite.

17. Specific objectives (should be written in bulleted form, a short paragraph indicating the methods to be followed for achieving the objective and verifiable indicators of progress should follow each specific objective).

- Identification of the proteases for their role in the parasite egg hatching process and targeting the genes coding for these proteases using RNAi approach.

- Delivery of siRNA / dsRNA molecules developed against these protease genes into the parasite eggs for establishing the precise role of these proteases in the egg hatching process.
18. Work Plan: should not exceed 3-4 pages (the section can be divided according to the specific aims and under each specific aim, the following should be stated clearly as sub headings).

18.1 Work plan (methodology/experimental design to accomplish the stated aim)

- **Designing of siRNA for gene silencing**
  - Gene sequences coding for various cathepsin-Ls and cathepsin-Bs as well as leucine aminopeptidases will be selected for designing siRNA / dsRNA against the consensus sequences of the different genes.
  - Expression sequence tags (ESTs) available in the database for *F. gigantica* will be screened for serine proteases using various bioinformatics tools and dsRNA will be designed against the identified sequence.

- **Synthesis of siRNA**
  - siRNA against these proteases will be synthesized *in vitro* using standard protocols. Double stranded RNA targeting the above protease genes will also be *in vitro* synthesized and evaluated for their silencing effect on the above genes. siRNA and dsRNA molecules will be evaluated for better silencing effect.

  - *In vitro* culture of *F. gigantica* eggs collected from the adult flukes will be set up for their embryonation and miracidial development.

**Delivery of the siRNA / dsRNA molecules to the parasite eggs**

Three different approaches will be used for delivering the siRNA / dsRNA molecules into the eggs at varying concentrations of these molecules.

- Direct soaking of the eggs in the siRNA /dsRNA molecules.
- Delivery of the siRNA / dsRNA into the eggs by electroporation.
- Use of a polymer or virus as a delivery vehicle for the transduction of these molecules in the eggs.

**Evaluation of the effect of RNAi on the expression of the targeted proteins**

- Gene silencing effect will be analyzed at the transcriptional level by quantitative real-time PCR of the above proteases genes, following standard protocols.
Antisera to *F. gigantica* recombinant cathepsin-L, cathepsin-B, leucine aminopeptidase and serine protease will be raised in rabbits. These antibodies will be used for probing the synthesis of the targeted proteins post-RNAi treatment in the embryonated eggs / miracidia by Western blotting and immuno-fluorescence, respectively.

- Confocal microscopy will be used for *in situ* localization of the targeted proteins in the developing embryo and the miracidium within the eggs.

- Inhibition of the egg hatching post-RNAi will be studied by recording the hatching percentage of the miracidia from the treated eggs to establish the role of the above proteases in the hatching process of the parasite eggs.

18.2 *Connectivity of the participating institutions and investigators* (in case of multi-institutional projects only)

NA

18.3 *Alternate strategies* (if the proposed experimental design or method does not work what is the alternate strategy).

Various standard gene silencing protocols of studying the gene function will be attempted, in case a single method fails to yield results.
<table>
<thead>
<tr>
<th>Period of study</th>
<th>Achievable targets</th>
</tr>
</thead>
</table>
| 6 Months      | siRNA /dsRNA molecules against the different alleles of cathepsin and leucine aminopeptidase genes in *F. gigantica* will be designed.  
Bioinformatics tools will be utilized for determining the *F. gigantica* ESTs coding for serine protease gene for designing siRNA against this target. |
| 12 Months     | Setting up of the *F.gigantica* in vitro egg culture for larval development.  
Delivery of the siRNA / dsRNA molecules into the eggs for studying their efficacy in the inhibition of larval hatching. |
| 18 Months     | Studying the effect of RNA interference on egg hatching at transcriptional level by standardization of quantitative Real-time PCR. |
| 24 Months     | Raising of antisera specific to *F. gigantica* recombinant proteins of cathepsin-L, cathepsin-B, serine protease and leucine aminopeptidase. |
| 30 Months     | Western blotting for studying the disruption of synthesis of targeted proteins post-RNAi.  
Confocal microscopy of the embryonated eggs / miracidia will be attempted for *in situ* localization of these proteins with in the larvae. |
<p>| 36 Months     | Data analysis, compilation of the results and report writing. |</p>
<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name</th>
<th>Designation</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dr. Y.D. Sharma</td>
<td>Professor and Head</td>
<td>Department of Biotechnology, AIIMS, Ansari Nagar, New Delhi-29. <a href="mailto:ydsharma_aiims@yahoo.com">ydsharma_aiims@yahoo.com</a></td>
</tr>
<tr>
<td>2.</td>
<td>Dr. Bhaskar Sharma</td>
<td>Principal Scientist(National Professor, ICAR)</td>
<td>Division of Veterinary Biochemistry, IVRI, Izatnagar, Bareilly, 243122 U.P <a href="mailto:bhaskar@ivri.res.in">bhaskar@ivri.res.in</a></td>
</tr>
<tr>
<td>3.</td>
<td>Dr. Utpal Tatu</td>
<td>Professor</td>
<td>Department of Biochemistry; Indian Institute of Science, Bangalore, Karnataka, 560012. <a href="mailto:tatu@biochem.iisc.ernet.in">tatu@biochem.iisc.ernet.in</a></td>
</tr>
<tr>
<td>4.</td>
<td>Dr. A.K. Tiwari</td>
<td>Principal Scientist</td>
<td>Division of Veterinary Biotechnology, IVRI, Izatnagar, Bareilly, U.P. 243122 <a href="mailto:aktiwari63@yahoo.com">aktiwari63@yahoo.com</a></td>
</tr>
<tr>
<td>5.</td>
<td>Dr. S.C. Yadav</td>
<td>Principal Scientist</td>
<td>National Research Centre on Equines, Sirsa Road, Hisar, Haryana. <a href="mailto:yadavsc@rediffmail.com">yadavsc@rediffmail.com</a></td>
</tr>
</tbody>
</table>
## PART IV: BUDGET PARTICULARS

### Budget (In Rupees)

#### A. Non-Recurring (e.g. equipments, accessories, etc.)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Item</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total Rs.Lakhs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gradient-Thermocycler with a compatible UPS</td>
<td>4.5</td>
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<tr>
<td>2</td>
<td>Electrophoresis Power Pack with electrophoresis assembly for DNA/protein gels</td>
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<td>0.0</td>
<td>0.0</td>
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<td>3</td>
<td>Laminar Flow hood (Clean air station)</td>
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<td></td>
<td>2.0</td>
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<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
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Sub total of A = Rs 8.0 lakhs

#### B. Recurring

##### B.1 Manpower (See guidelines at Annexure-III)

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<th>S.No.</th>
<th>Position No.</th>
<th>Consolidated Emolument</th>
<th>Year 1 Rs lakhs</th>
<th>Year 2 Rs lakhs</th>
<th>Year 3 Rs lakhs</th>
<th>Total Rs lakhs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Senior Research Fellow</td>
<td>Rs.16,000 +20% HRA / month 19,200/= first year)</td>
<td>2.3 @ Rs.19,200/ month</td>
<td>2.5 @ Rs.21,400/ month</td>
<td>2.8 @ Rs.23,400/ month</td>
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Sub-Total (B.1) = Rs 7.6 lakhs
### B.2 Consumables

<table>
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<tr>
<th>Item</th>
<th>Quantity</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total Rs lakhs</th>
</tr>
</thead>
<tbody>
<tr>
<td>siRNA / ds RNA synthesis kits, Real-time PCR kit, recombinant protein expression reagents, Western blotting and confocal microscopy reagents.</td>
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<td>7.0</td>
<td>7.0</td>
<td>3.0</td>
<td>17.0</td>
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</tbody>
</table>

Sub-Total (B-2) = Rs 17.0

**Note:** Please give justification for each head and sub-head separately mentioned in the above table.

Financial Year: April - March

In case of multi-institutional project, the budget estimate to be given separately for each institution.
PART V: EXISTING FACILITIES
Resources and additional information

1. Laboratory: Laboratory facilities required for the proposed research work are available.

   b. Equipments. Equipments, other than those requested in the project proposal are available.

2. Other resources such as clinical material, animal house facility, glass house, Experimental garden, pilot plant facility etc.
   NA

<table>
<thead>
<tr>
<th>Other items</th>
<th>Consolidated Emolument</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total Rs lakhs</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.3 Travel</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>B.4 Contingency (including AMC of the existing equipments in the lab to be used in the project work).</td>
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<td>3.0</td>
<td>2.0</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>B.5 Overhead (If applicable)</td>
<td>10% of the total budget (excluding the equipment cost).</td>
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<td>1.5</td>
<td>0.5</td>
<td>3.5</td>
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<tr>
<td>Sub–total (B3+B4+B5)</td>
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<td></td>
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<td>13.0</td>
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<tr>
<td>Sub-total of B (B.1+B.2+B.3+B.4+B.5)</td>
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<td>Grand Total (A + B)</td>
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<td>45.6</td>
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</tbody>
</table>
PART VI: DECLARATION/CERTIFICATION

a) the research work proposed in the scheme/project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b) the same project proposal has not been submitted to any other agency for financial support.

c) the emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/university or as per the Ministry of Science & Technology guidelines (Annexure-III)

d) necessary provision for the scheme/project will be made in the Institute/University/State budget in anticipation of the sanction of the scheme/project.

e) if the project involves the utilisation of genetically engineered organisms, we agree to submit an application through our Institutional Biosafety Committee. We also declare that while conducting experiments, the Biosafety Guidelines of the Department of Biotechnology would be followed in toto.

f) if the project involves field trials/experiments/exchange of specimens, etc. we will ensure that ethical clearances would be taken from concerned ethical Committees/Competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g) it is agreed that any research outcome or intellectual property right(s) on the invention(s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance, Department of Expenditure, as contained in Annexure-V.

h) we agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i) the institute/university agrees that the equipment, other basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended to investigator(s) throughout the duration of the project.

j) the Institute assumes to undertake the financial and other management responsibilities of the project.
Signature of Project Coordinator
(applicable only for multi-institutional projects)
Date:

Signature of Principal Investigator:
Date: 26.9.11

Signature of Co-Investigator
Date:

Signature of Executive Authority of Institute/University with seal
Date:

Director
Indian Veterinary Research Institute
IZATNAGAR-244122, (U.P.) India

Signature of Co-Investigator
Date:
Name: O. K. Raina
Designation: Senior Scientist
Department /Institute: Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., India
Date of Birth: 20-04-1958 Sex (M/F) Male SC/ST: General

**Education** (Post-Graduation onwards & Professional Career).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Institution/place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>University of Kashmir, Srinagar, J&amp; K</td>
<td>M. Sc. Zoology</td>
<td>1981</td>
<td>Zoology(with specialization in Parasitology)</td>
</tr>
<tr>
<td>2</td>
<td>Do</td>
<td>M.Phil (Parasitology)</td>
<td>1983</td>
<td>Helminthology</td>
</tr>
<tr>
<td>3</td>
<td>All India Institute of Medical Sciences, New Delhi</td>
<td>Ph.D (Biotechnology)</td>
<td>1999</td>
<td>Characterization of knob protein of <em>Plasmodium falciparum</em>.</td>
</tr>
</tbody>
</table>
A. Position and Honors

Position and Employment (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>Institution Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P</td>
<td>Senior Scientist</td>
<td>June 2002</td>
<td>Continuing</td>
</tr>
<tr>
<td>Do</td>
<td>Scientist</td>
<td>1998</td>
<td>2002</td>
</tr>
<tr>
<td>Do</td>
<td>Scientist</td>
<td>June 1993</td>
<td>1998</td>
</tr>
<tr>
<td>Do</td>
<td>Technical Officer(T-6)</td>
<td>Feb.1989</td>
<td>June-1993</td>
</tr>
</tbody>
</table>

B. Publications (Numbers only): 45
Books: One Book chapter.  
Research Papers: 45
General articles: 4
Patents: Nil
Others (Please specify): Nil

Selected peer-reviewed publications on Fasciola gigantica


C. Research Support

**Ongoing Research Projects**

<table>
<thead>
<tr>
<th>S No</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Study of herbal acaricides as means to overcome the development of resistance in ticks to conventional acaricides. <em>(Working as associate).</em></td>
<td>ICAR, New Delhi (NAIP;ICAR) , New Delhi</td>
<td>389 lakhs</td>
<td>2008-2012</td>
</tr>
</tbody>
</table>

**Completed Research Projects** (State only major projects of last 3 years)
<table>
<thead>
<tr>
<th>S.No</th>
<th>Title of the Project</th>
<th>Funding agency</th>
<th>Amount</th>
<th>Date of completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Immuno-prophylactic evaluation of some potential vaccine candidates of <em>Fasciola gigantica</em> in domestic animals.</td>
<td>DBT, New Delhi.</td>
<td>35.03 lakhs</td>
<td>Sep.2007-Feb.2010</td>
</tr>
</tbody>
</table>

Place: Izatnagar  
Date: 08-09-2011  
Signature of the Investigator
Co-Investigator- I

Name: Dr Praveen K. Gupta Designation: Senior Scientist
Institute: Indian Veterinary Research Institute
Email: praveen@ivri.res.in
Fax: 0581-2301584
Ph: 0581-2301584(O), 09456408509 (M)
Date of Birth: 27th August 1965 Sex (M/F): M

Education:

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Institution Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G.B. Pant Univ. Ag &amp; Tech Pantnagar</td>
<td>B.V.Sc. &amp; A.H.</td>
<td>1988</td>
<td>Veterinary Sciences</td>
</tr>
<tr>
<td>2.</td>
<td>IVRI, Izatnagar</td>
<td>M.V.Sc. (Animal Biotechnology)</td>
<td>1991</td>
<td>Animal Biotechnology</td>
</tr>
<tr>
<td>3.</td>
<td>G.B. Pant Univ. Ag &amp; Tech Pantnagar</td>
<td>Ph.D. (Molecular Biology &amp; Biotechnology)</td>
<td>1998</td>
<td>Molecular Biology, Genetic Engineering and Biotechnology</td>
</tr>
</tbody>
</table>

D. A. Position and Honors

Position and Employment:

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Institution Place</th>
<th>Position held</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>IVRI, Izatnagar</td>
<td>Senior Scientist</td>
<td>3rd June 2002- till date</td>
</tr>
<tr>
<td>2.</td>
<td>IVRI, Izatnagar</td>
<td>Scientist (SS)</td>
<td>3rd June 1998-2nd June 2002</td>
</tr>
</tbody>
</table>

B. Publications:

Research Papers: 70
General articles: 04
GenBank Submissions: 35
Training programmes conducted: 2
Students Supervised/Guided: 11

Selected peer-reviewed publications:


**Research Support**

**C. Ongoing DBT Research Project**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount (Rs)</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Evaluation of anti-rabies effect of small interfering RNA (siRNA) delivered through viral vector</td>
<td>Department of Biotechnology, GOI</td>
<td>56.65 lakhs</td>
<td>June 2009, 3 years</td>
</tr>
</tbody>
</table>

**Completed DBT Research Projects:**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount (Rs)</th>
<th>Date of completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Development of RNA replicon-based vaccine vector for enhanced expression and immune responses</td>
<td>Department of Biotechnology, GOI</td>
<td>23.03 lakhs</td>
<td>Development of RNA replicon-based vaccine vector for enhanced expression and immune responses</td>
</tr>
</tbody>
</table>

Place: Izatnagar
Date: 7th September 2011

(Praveen K Gupta)
Signature of Co-Investigator
Co-Investigator II

Designation: **Principal Scientist**

Department/Institute/University: **Biochemistry, IVRI, Izatnagar**

Date of Birth: **20th August 1956**, Sex (M/F) **Male**  SC/ST: **Not Applicable**

**Education** (Post-Graduation onwards & Professional Career)

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution Place</th>
<th>Degree Awarded</th>
<th>Year</th>
<th>Field of Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.S. University, Baroda</td>
<td>Ph.D.</td>
<td>1988</td>
<td>Biochemistry of Malarial Parasite</td>
</tr>
<tr>
<td>2</td>
<td>M.S. University, Baroda</td>
<td>M. Sc.</td>
<td>1977</td>
<td>Biochemistry</td>
</tr>
</tbody>
</table>

**E. Position and Honors**

**Position and Employment** (Starting with the most recent employment)

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Institution Place</th>
<th>Position</th>
<th>From (Date)</th>
<th>To (date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Biochemistry, IVRI, Izatnagar</td>
<td>Principal Scientist</td>
<td>January 1998 (July)</td>
<td>To date</td>
</tr>
<tr>
<td>2</td>
<td>Biochemistry, IVRI</td>
<td>Senior Scientist</td>
<td>1986 (Jan)</td>
<td>1998 (Jun)</td>
</tr>
<tr>
<td>3</td>
<td>Biochemistry</td>
<td>Scientist S 2</td>
<td>1984 (July)</td>
<td>1985(Dec)</td>
</tr>
<tr>
<td>4</td>
<td>Biochemistry</td>
<td>Scientist S 1</td>
<td>1978(Aug)</td>
<td>1984(July)</td>
</tr>
</tbody>
</table>

**Honors/Awards**

Young Scientist Gold Medal at the Asian Congress of Parasitology (1986), Overseas Fellowship supported by NIH grant (1990-92), Institute award of Honour (2002) for research contribution

**Professional Experience and Training relevant to the Project**

Worked as a research Fellow at CDRI, Lucknow (1983-86) on malarial parasite, supported by WHO; Research Associate at Duke University Medical Ctr., Durham, (USA) (1990-92) ñ worked on Protein Structure/Function relationships

**B. Publications** (Numbers only)

Books : ...................... Research Papers: **36**. General articles: **Four**


List maximum of five recent publications relevant to the proposed area of work.


F. Research Support

Ongoing Research Projects

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Title of Project</th>
<th>Funding Agency</th>
<th>Amount</th>
<th>Date of sanction and Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Complement C3 binding protein of Haemonchus contortus and its significance in host-parasite interactions</td>
<td>DBT</td>
<td>Rs 34.0 lakh</td>
<td>September 2010 (3 years)</td>
</tr>
<tr>
<td>2</td>
<td>The role of extracellular matrix proteins vitronectin and fibronectin in the establishment of staphylococcus aureus infection.</td>
<td>ICMR</td>
<td>Rs 18.13 Lakh</td>
<td>November 2011(3 years)</td>
</tr>
<tr>
<td>No.</td>
<td>Title of Project</td>
<td>Funding Agency</td>
<td>Amount</td>
<td>Date of completion</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>1</td>
<td>Molecular &amp; functional --- <em>H. contortus</em>.</td>
<td>DBT Institute</td>
<td>Rs 24 Lakh</td>
<td>2006</td>
</tr>
<tr>
<td>2</td>
<td>Evaluation of C1q binding -- ---- <em>H. contortus</em> calreticulin.</td>
<td>DBT Institute</td>
<td>Rs 5.0 Lakh (contingency only)</td>
<td>2008</td>
</tr>
</tbody>
</table>