

# **Evaluation of immunological response to rabies vaccination in pet dogs and KAP assessment of their owners**

**BY**  
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**(J-15-MV-431)**

**Thesis submitted to**  
**Faculty of Postgraduate Studies**  
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**IN**  
**VETERINARY PUBLIC HEALTH AND EPIDEMIOLOGY**




**Division of Veterinary Public Health and Epidemiology**  
**Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu**  
**Main Campus, Chatha, Jammu – 180009**  
**2017**

## CERTIFICATE -I

This is to certify that the thesis entitled "Evaluation of Immunological response to rabies vaccination in Pet Dogs and KAP Assessment of their owners" submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science in Veterinary Public Health and Epidemiology** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu is record of bonafide research, carried out by **Ms. Suhasani Tandon**, Registration No. **J-15-MV-431** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

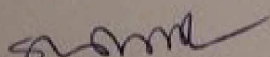
It is further certified that help or assistance received during the course of the thesis investigations have been duly acknowledged.

  
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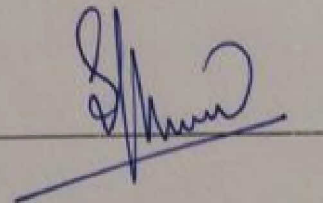
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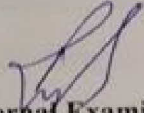
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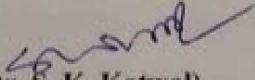
  
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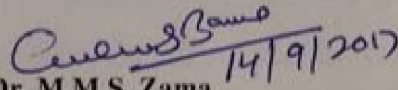
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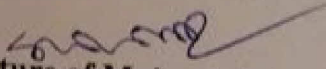
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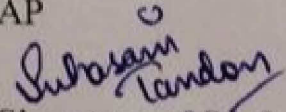
## ABSTRACT

<b>Title of Thesis</b>	: <b>EVALUATION OF IMMUNOLOGICAL RESPONSE TO RABIES VACCINATION IN PET DOGS AND KAP ASSESSMENT OF THEIR OWNERS</b>
<b>Name of Student</b>	: Suhasani Tandon
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<b>Name and Designation of Major Subject</b>	: Dr. S.K. Kotwal, Professor & Head Division of Veterinary Public Health and Epidemiology, F.V.Sc & A.H., R.S. Pura
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Study assessed prevalence of anti rabies antibodies in vaccinated dog sera (180) in and around Jammu by ELISA and carried Knowledge, Attitude and Practices (KAP) of dog owners (rural/urban). Qualitatively assayed sero-prevalence was 56.43 percent in different age groups. Predominant sero-prevalence was recorded in category III (yearly booster vaccination). Quantitatively assayed 43 positive samples revealed predominant sero -prevalence (61.55%) in category III dogs. Overall sero-prevalence being 49.44 and 55.81 percent qualitatively and quantitatively respectively. Protective titre  $\geq 0.5\text{IU}/\mu\text{l}$  was recorded in 34/43 (79.06%) sera samples. Annual boosters offered better protection so recommended for field vaccination practices. KAP analysis using pre-tested and structured questionnaires on 200 respondents interviewed comprised urban 130 (65%) and rural 70 (35%) respondents. Respondents 157(76%) were males and 48(24%) females with maximum from age group of 18-29 years (55%). Respondents with 77.8 and 77 percent had heard about rabies and its fatal consequences respectively. Significant results observed in knowledge and attitude vis-a-vis dog ownership. Urban population (98.9 %) believed rabies to be fatal, transmitted by dogs or other species (98.9%). Besides, 94 percent believed that owned dog must receive antirabies vaccine, 89-94 percent believed reporting to hospital or higher authority about dog bite and outbreak of rabies in community whereas, 78 percent believed to kill rabies suspected stray dog. Dog owners followed good practices compared to non-dog owners. KAP w.r.t prevention and treatment of rabies were inadequate amongst rural population necessitating community based health education and rabies awareness in these areas.

**Keywords:** Vaccination, Antirabies antibodies, Canines, ELISA, KAP

  
Signature of Major Advisor

  
Signature of Student

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## ABBREVIATIONS

%	-	Percent
Avg.	-	Average
DW	-	Distilled Water
EB	-	ELISA Buffer
<i>et al.,</i>	-	<i>et alii/ Alia</i> (any other people)
F	-	Female
FAO	-	Food and Agricultural Organization
HRPO	-	Horse Radish Peroxidase
pH	-	Hydrogen ion concentration
J&K	-	Jammu and Kashmir
KAP	-	Knowledge, Attitude and Practice
M	-	Male
MANOVA	-	Multivariate Analysis of Variance
ICAR	-	Indian Council of Agricultural Research
ELISA	-	Enzyme linked Immunosorbant Assay
OD	-	Optical Density
OIE	-	Office International des Epizooties
WHO	-	World Health Organization
S.No.	-	Serial Number
Rpm	-	Revolutions per minute
5X	-	five times
@	-	at the rate of
S/P	-	Samples/Positive
MV	-	Mean values

OD	-	Optical density
PC	-	Positive control
NC	-	Negative control
FAVN	-	Fluorescent Antibody Virus Neutralization
VN	-	Virus Neutralization
RFFIT	-	Rapid Fluorescent Focus Inhibition Test
NT	-	Neutralization
MH	-	Mixed Hemadsorption
IF	-	Immuno Fluorescence
CVS	-	Challenge Virus Standard
FAT	-	Fluorescent Antibody Test
MIT	-	Mouse Inoculation Test

### **Unit of Measurement**

μl	-	Micro litre
°C	-	Degree Celsius
Hr.	-	Hour
IU	-	International Unit
min	-	minute
ml	-	Millilitre
nm	-	Nanometre
No.	-	Number
Yr	-	Year
>	-	Less than
<	-	Greater than
≥	-	Less than equal to
≤	-	Greater than equal to

Chapter-I

# Introduction

Rabies is a serious viral zoonoses that is responsible for over 50,000 human mortalities annually worldwide. Most of these occurring mainly in the developing world, especially in Asia (56%) and Africa (44%) (WHO, 2005). The rabies virus is readily transmitted between mammals, whether they are of the same or different species. Less often, an animal or person is infected by contact with infectious saliva or neurological tissues, through mucous membranes or breaks in the skin. The rabies virus may not be transmitted through intact skin. A few cases have been reported after transplantation of organs, particularly corneas but also pancreas, kidneys and liver. Aerosol transmission has been documented in special circumstances, such as in laboratories and bat caves with an unusually high density of aerosolized, viable virus particles. Rabies viruses have been transmitted by ingestion in experimentally infected animals (OIE, 2009).

Rabies is a neurological disease of mammals that is almost invariably fatal once the clinical signs develop. It is an acute, viral encephalomyelitis which affects all warm blooded animals with mortality rate being close to 100 percent (Vegad *et al.*, 2008). Globally, 2 persons die every hour due to rabies of which 40% of people who are bitten by suspect rabid animals are children under the age of 15 years (WHO, 2006). Rabies is caused by neurotropic virus of the genus *Lyssavirus* in the family of Rhabdoviridae, and is transmissible to all mammals, so all suspected material must be handled under the appropriate safety conditions as specified by World Health Organisation (WHO,1996). Clinical diagnosis of encephalitis is challenging, and all suspected and probable clinical cases of rabies should be confirmed by laboratory tests. Each country should have national reference laboratory with the capacity for basic rabies diagnosis and case confirmation by modern techniques (Barrat *et al.*, 2006). WHO, the World Organization for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and the Global Alliance for Rabies Control have set a global target of “zero human rabies deaths by 2030” (WHO, 2017).

Rabies is maintained in two epidemiological cycles, one urban and one sylvatic. In the urban rabies cycle, dogs are the main reservoir host. This cycle predominates in areas of Africa, Asia, and Central and South America where the proportion of unvaccinated and semi-owned or stray dogs is high. The sylvatic (or wildlife) cycle is the predominant cycle in Europe and North America. It is also present simultaneously with the urban cycle in some parts of the world. The epidemiology of this cycle is complex; factors affecting it include the virus strain, the behavior of the host species, ecology and environmental factors (OIE, 2009).

Over 99 percent of human exposures to rabies result from the bite of domestic dog (*Canis familiaris*) where there is increase in dog population and prevalent poor dog ownership practices prevail (Adaba *et al.*, 2004). In many instances, the management system is very poor and dogs are allowed to roam and search for food and shelter, consequently increase in cases of dog bite, potentially exposing humans to rabies. Most dogs are not vaccinated against this vaccine preventable disease (Ahmed *et al.*, 2000). Voluntary vaccination of dogs is declining and rarely they are tested for immune response. WHO recommends that about 70 percent of dogs need to be vaccinated to control rabies in a community (WHO, 1989; Knobel *et al.*, 2007). It is not enough just to vaccinate individual dogs, rather, the efficiency of the vaccines evidenced by sero-conversion need to be monitored. It is possible to estimate the vaccine efficiency by various available serological tests for specific antibodies to lyssaviruses (Trimarchi and Nadin-Davis, 2007).

The ELISA provides a rapid (approximately 4 hours) test that avoids the requirement to handle live rabies virus. Commercial indirect ELISA kits are available that allow detection of rabies antibodies in individual dog and cat serum samples following vaccination. In 2007, the International Committee adopted such methods as Prescribed Tests for evaluating vaccine responses in dogs and cats prior to international movement, provided a kit is used that has been validated and adopted on the OIE Register as fit for such specific purposes.

Keeping in view the fatal nature of disease, it becomes imperative to assess the immunological response to rabies vaccination vis-a-vis the protective titre. As such it is proposed to study these facets. This study was carried out to determine the prevalence of antibodies to rabies in vaccinated pet dogs by indirect ELISA.

Knowledge, Attitude and Practice (KAP) study as a cross sectional study helps in assessing the status of dog owners pertaining their knowledge about rabies. In countries where the virus circulates in the dog population, more than 99 percent of all human rabies cases are the result of exposure to rabid dogs (Wandeler *et al.*, 1993). A person bitten by a rabid dog, if untreated with post-exposure prophylaxis (PEP), has about a 5 percent (if bitten on hand) to 70 percent (if bitten on face) probability of developing clinical rabies (Cleaveland *et al.*, 2002). With one exception, clinical cases of human rabies are always fatal (Willoughby *et al.*, 2005). The awareness of mode of rabies transmission, associated rabies risk, vis-a-vis adequate preventive measures needs to be assessed (Altmann *et al.*, 2008). Efforts to educate the public about the epidemiological features of rabies and simple “Dos and Don’ts” that can protect them as well as help in bringing about a reduction in the incidence of rabies are nil (WHO, 2004).

Studies on knowledge, attitude and practice (KAP) towards rabies among dog meat processors and dog meat consumers have been carried out in different parts of the Nigeria (Opaleye *et al.*, 2006; Isek, 2013; Odeh *et al.*, 2014) and they have recognized KAP as an important factor in the control of rabies. A KAP study on rabies in Kandy District, Central Province, showed that there was high level of awareness and receptiveness to rabies control measures among the people (Matibag *et al.*, 2007). The practices that need to be improved include: reporting of dog bites; knowledge and enthusiasm to take suspected animals to a diagnostic laboratory for disease confirmation; seeking medical treatment after a dog bite; pet restriction and vaccination.

In India, there are very few community-based epidemiological studies on knowledge, attitude and practice about rabies prevention and control (Prakash *et al.*, 2012). The questionnaire based information on demographic characteristics of the dog owners, their association with dogs, knowledge, attitude and practice towards rabies were

evaluated in the present study. Associations between demographic variables and KAP scores were assessed by using appropriate statistical tests (Veronica *et al.*, 2014).

In Jammu, the studies on evaluation of immunological response in dogs against anti-rabies vaccination as well as KAP of the dog owners have not been conducted. Presented study facets might lead to the future awareness regarding vaccination of the pet dogs and status of the control and prevention of Rabies amongst both dogs and humans who are in contact with them. The conducted study throws light upon on the protective levels of the vaccines used and if not achieved desired protection, then what might be the probable causes. The KAP help in enlightening with the various lacunas amongst respondents who were interviewed under the study. So, the present study was taken up to generate data for evaluating such responses in order to check the status with following objectives:

1. To evaluate immune response to rabies vaccination in pet dogs.
2. To assess Knowledge, Attitude and Practice (KAP) of Dog owners to canine rabies in region of Jammu.

Chapter-II

Review  
of  
Literature

## CHAPTER-II

## REVIEW OF LITERATURE

---

The rabies prophylactic vaccine is given as 3-5 doses all over the world and it is usually administered intramuscularly or subcutaneously. Fewer doses with no rabies immunoglobulin are given if the animal was previously vaccinated. Post exposure prophylaxis is highly effective if it is begun soon after exposure (OIE, 2011). There is no effective treatment once the symptoms develop. Vaccines, antiviral drugs such as ribavirin, interferon-alpha, passively administered anti-rabies virus antibodies (monoclonal antibodies), ketamine and/or the induction of a coma have been tried in the past, but were usually ineffective (OIE, 2009).

The main application of serology for classical rabies is to determine responses to vaccination, either in domestic animals prior to international travel, or in wildlife populations following oral immunization. In accordance with the WHO recommendations (WHO, 1985), 0.5 IU per  $\mu\text{l}$  of rabies antibodies is the minimum measurable antibody titre considered to represent a level of immunity in humans that correlates with the ability to protect against rabies infection. The same measure is used in dogs and cats to confirm a satisfactory response to vaccination. As neutralizing antibodies are considered a key component of the adaptive immune response against rabies virus (Hooper *et al.*, 1998) the gold standard tests are virus neutralization (VN) tests. However, indirect ELISA's have been developed that do not require high-containment facilities and produce rapid results. Care should be taken when correlating results between virus neutralization tests and ELISA's owing to the inherent differences between them. Multiple publications demonstrate a variable sensitivity and specificity for ELISA's in both humans and animals (Bedeckovic *et al.*, 2016; Morshedi and Aslani, 2002; Fachin *et al.*, 2005; Feyssalguet *et al.*, 2006; Servat *et al.*, 2006). Although VN tests are recommended where specific assessment of protection is required, both tests are useful for detecting responses to vaccination if appropriate cut-offs are used.

An ELISA that detects rabies antigen is a variation of the immunochemical test. It is useful for large epidemiological surveys. However, the specificity and sensitivity of

such tests for locally predominant virus variants should be checked before use (Xu *et al.*, 2007).

The ELISA provides a rapid (approximately 4 hours) test that avoids the requirement to handle live rabies virus. Commercial indirect ELISA kits are available that allow detection of rabies antibodies in individual dog and cat serum samples following vaccination.

The prevention and control of rabies is usually a national responsibility and, in many countries, the vaccine may be used only under the control of the Competent Authority. Virulent rabies virus may be used to produce inactivated rabies vaccine; consequently, the rabies vaccine production facility should operate under the appropriate biosafety procedures and practices. The facility should meet the requirements for containment outlined by WHO (2005).

Rabies vaccines are defined as a standardised formulation containing defined amounts of immunogens. These immunogens are either inactivated (killed), live-attenuated or biotechnology-derived. Licensed vaccines for the parenteral vaccination of domestic animals and oral vaccines for the immunisation of wild animals are available. These vaccines are frequently used off-label in captive and free-ranging wild animals. Oral vaccines for dogs are in the experimental stage (WHO, 2007).

Rabies is not regarded as a candidate for global eradication (Rupprecht *et al.*, 2008). From a global public health perspective however, the dog should be considered a main target for rabies elimination as it is the principal reservoir. The disease can also be successfully controlled in certain wild carnivores, such as red foxes and raccoon dogs (Cliquet *et al.*, 2012). Apart from dogs, other companion animals (such as cats, ferrets, etc.) and livestock pose a risk for human exposure and would benefit from inclusion in any national vaccination programme. Additionally, vaccination of livestock is recommended as it secures livelihoods in many parts of the world.

The studies on Knowledge, Attitude and Practices (KAP) regarding rabies amongst dog owners have been conducted earlier in different parts of the world (Opaleye *et al.*, 2006; Isek, 2013; Odeh *et al.*, 2014; Ajayi *et al.*, 2006; Fagbami *et al.*, 1981;

Altmann *et al.*, 2008) and they have recognized KAP as an important factor in control and prevention of rabies. In rabies endemic countries, upto 99 percent of humans rabies cases are due to exposure through rabid dogs (Wandeler *et al.*, 1993). WHO (2004) suggested to excavate the efforts regarding awareness/education of the people about rabies and bringing out the lower incidence rate of rabies.

The presented study facets regarding evaluation of immunological response in dogs against anti-rabies vaccination and KAP assessment of dog owners have not been conducted earlier in the region of Jammu. Similar works reportedly conducted elsewhere, whether in India or in world have been reviewed in the afore detailed paragraphs.

## **2.1 IMMUNOLOGICAL TESTS USED FOR EVALUATION OF IMMUNE RESPONSE TO RABIES VACCINATION**

Webster and Dawson (1935) devised an *in vivo* test for determining rabies virus neutralization (VN) antibodies: dilutions of sera are mixed with a constant virus dose, incubated for 1.5 hours, and inoculated intracerebrally into weanling mice. . This has been the accepted and most common procedure for testing rabies VN antibody (Atanasiu, 1966), although it is an expensive one with the inherent disadvantage of a 2-week interim between inoculation and final results. Moreover, variability in mouse susceptibility may affect the results (Johnson and Leach, 1940).

Atanasiu and Lepine (1959) described that the cytopathic effect with rabies had not been routinely reproducible, and the tissue culture (TC) neutralization techniques used in other viral diseases, such as metabolic inhibition in poliomyelitis (Salk *et al.*, 1954) or cytopathic effect in monolayer for measles (Enders and Peebles, 1954), have not been readily applicable to this disease. In recent years, however, several laboratories have developed *in vitro* techniques for rabies VN testing.

Smith *et al.* (1973) in USA conducted Rapid Fluorescent Focus Inhibition Test (RFFIT), a rapid reproducible test for determining rabies neutralizing antibody. BHK-21 cells were infected with tissue culture-adapted rabies virus. The results, obtained in 24 hours, were comparable with those of the standard mouse neutralization test.

Monica (1976) in Sweden assessed Humoral antibody response to three types of rabies vaccines assayed by the neutralization (NT), the mixed hemadsorption (MH), and the indirect immunofluorescence (IF) tests. The NT and MH tests were used to detect antibodies combining with antigens at the surface of virions and infected cells, whereas the indirect IF test measured antibodies mainly to the rabies nucleocapsid antigen. After immunization with a human diploid cell vaccine, antibodies were detected by both the NT and the MH test in the 14th- and 30th-day serum samples from each of eight vaccinated persons. Antibodies elicited by duck embryo and nervous tissue vaccines occurred less frequently and in lower titers. In these groups of vaccines, 5 of 14 and 5 of 10, respectively, had antibodies detectable by the NT test in the 14th- and 30th-day sera but were negative by the MH test. Antibody titers which they obtained by the indirect IF test in the human diploid cell vaccine group were relatively lower compared to the duck embryo and nervous tissue vaccine groups.

Briggs *et al.* (1998) studied a comparison of RFFIT and FAVN (Fluorescent Antibody Virus Neutralization) test for detecting immune response after rabies vaccination in dogs and cats being exported to Rabies-free areas. Test to test variation reportedly occurred in results obtained by both RFFIT and FAVN for 95 sera from vaccinated animals with low to moderate levels of neutralizing antibody (RFFIT titre <1.0 IU). No significant differences (p-value = 0.43) were detected for the 95 sera in the frequency for one test with respect to the other to have a positive response ( $\geq 0.5$  IU) or the marginal homogeneity (p-value = 0.39) of results obtained by the two methods.

Muhamuda *et al.* (2007) in Bangalore, India conducted a study to evaluate competitive ELISA for estimation of rabies neutralizing antibodies after post-exposure rabies vaccination in humans. All the serum samples that were positive by RFFIT were also found positive by C-ELISA. The titers obtained with C-ELISA were marginally higher than the RFFIT titers, but a significant correlation was noted between the two tests ( $r = 0.897$ ). None of the negative controls were detected to be positive for rabies antibodies by either of these tests. On the basis of results, the C-ELISA was found to be 100 percent specific and sensitive in comparison to RFFIT.

Kramer *et al.* (2009) in Europe used RFFIT for batch potency testing of inactivated rabies vaccines. A comparative study was performed in which they demonstrated a good correlation between the challenge test results and the mean titers determined by RFFIT. Furthermore, all the vaccine batches failing challenge test were also recognized as insufficient in the serological assay.

Rad *et al.* (2010) in Iran studied a comparative analysis of antibody titres produced by two BHK rabies vaccines in field trial experiment condition in dogs by RFFIT. Two BHK-rabies vaccines were tested on 12 dogs divided into two groups. Sera were taken monthly over 18 months to evaluate the titer of the rabies neutralizing antibody by the rapid fluorescence focus inhibition test (RFFIT) at the end of each month. Both study groups reportedly showed a sufficient immunogenic response without any significant difference for at least one year after first vaccination.. According to the results, at the end of the study (18<sup>th</sup> month) serum titer in only one dog (No. 7) was less than protective level. Two animals in group one (No. 2 and No. 5) also had serum titers less than protective level. Mean of post vaccination antibody titer were not different in either groups (P=0.35). They concluded that with respect to the effective immunization of rabies vaccines, their annual injections would be sufficient.

Dzikwi *et al.* (2010) conducted a study to determine vaccination and immune status of owned dogs in Zaire. Accompanying information obtained included the vaccination status of the dogs, sex, location and management system. The samples were tested for virus neutralising antibodies by the rapid fluorescence focus inhibition test (RFFIT) using the challenge virus standard (CVS-11 strain). Only 32 (16.9%) of the dogs in their study had a history of vaccination of which 23 (71.9%) had virus neutralizing antibodies (VNA) to CVS-11. Five dogs reportedly vaccinated had no demonstrable VNA and 20 (14.8%) had VNA even though they were said to be unvaccinated by their owners.

Ondejkova *et al.* (2015) compared the results of determination of rabies antibodies using three *in vitro* methods: rapid fluorescent focus inhibition test (RFFIT), fluorescent antibody virus neutralization test (FAVNT) and the immune enzymatic assay (ELISA). Sera with low and sufficiently high titre of antibodies had a similar result in all

the methods. According to the experiment a critical level of rabies antibodies close to the required protection level (0.5 IU/ $\mu$ l) was reported in sera of 18 dogs (9.18%); collected after primary vaccination of dogs.

Mani *et al.* (2013) in Bangalore, India presented a review article on laboratory diagnosis of Human Rabies in which they suggested rapid diagnosis of rabies might help initiate prompt infection control and public health measures, obviate the need for unnecessary treatment/medical tests, and assist in timely administration of pre- or post exposure prophylactic vaccination to family members and medical staff. Traditional methods for ante-mortem and post-mortem rabies diagnosis were reported to have several limitations. However, the recent advances in technology have led to improvement or development of several diagnostic assays for rabies viral antigen and antibody detection and assays for viral nucleic acid detection and identification of specific biomarkers. They suggested that these assays which complement traditional methods had the potential to revolutionize rabies diagnosis in future.

### **2.3 MASS VACCINATION AGAINST RABIES AND ITS EVALUATION**

Adeyemi and Zessin (2000) in Nigeria recorded cases of 755 dogs presented in the Small Animal and Preventive Veterinary Medicine Clinics of the University of Ibadan for analyzing anti-rabies vaccination coverage and booster immunization consistency. Two hundred and seventy-six (149 cases of primary vaccination and 84 cases of booster vaccination) of the total 755 registered dogs were reportedly vaccinated against rabies during the 5-year period. Inadequate vaccination coverage, urban rabies control problems and public health implications in Ibadan, the largest city in West Africa, were discussed.

Durr *et al.* (2009) investigated the percentage of dogs that could be vaccinated against rabies by conducting a pilot campaign in N'Djame'na, Chad. Owners were charged US\$4.13 per dog vaccination, and 24 percent of all dogs in the three city districts covered by the campaign were vaccinated. Total campaign costs were US\$7623, with an average of US\$19.40 per vaccinated dog. This being five times more expensive than the cost per animal vaccinated during a previous free vaccination campaign for dog-owners, conducted in the same districts. The free campaign, which vaccinated 2605 more dogs

than this campaign, cost an additional US\$1.45 per extra dog vaccinated. Reported that the campaigns in which owners were charged for vaccination, resulted in lower vaccination rate than in free campaigns. They recommended that the public health officials can use these results while evaluating the costs and benefits of subsidizing dog rabies vaccination programmes.

Knoop *et al.* (2010) in Germany conducted a study to evaluate a commercial rabies ELISA as an alternative to serum neutralization assays as part of the pet travel scheme and oral vaccination campaigns of foxes. When dog and cat sera were analyzed by ELISA, the sensitivity compared to the standard serological test of 36.9–82.0 percent and 44.4–88.9 percent, respectively, was calculated depending on the method used. Reportedly, for fox field samples from oral vaccination areas the sensitivity compared to the Rapid Fluorescent Focus Inhibition Test (RFFIT) was 32.4 percent (95% CI 24.8–40.0%). They observed that in the present format ELISA (which they used) cannot replace standard serological assays neither in the pet travel scheme nor in follow-up investigations for oral vaccination campaigns.

Ilieva (2013) reported the findings of a study to assess the efficiency of oral vaccination against rabies in the fox population in Bulgaria. In this study, a total of 3,321,380 baits including vaccine doses (Lysvulpen modified live vaccine) and tetracycline were disseminated over 19 districts (55,995 sq. km area) mainly in Northern and Western Bulgaria during 3 vaccination campaigns. The efficiency of oral vaccination was assessed in shot foxes directly by measurement of the circulating anti-rabies antibodies with a specific ELISA test and indirectly by determining tetracycline accumulation in teeth. Concomitantly, the presence of the wild rabies virus was investigated in brain samples from various animals with evocated clinical signs or dead (including shot foxes) using fluorescent antibody test (FAT) and mouse inoculation test (MIT) during 2007-2010. Reported that the sero-conversion rates in the fox population were 18.3 percent and 17.0 percent in 2009 and 2010, respectively and the frequencies of tetracycline accumulation in teeth were 11.1 percent and 12.0 percent. Only 7 foxes in 2009 and 4 in 2010 reportedly tested positive by these two methods.

Jibat *et al.* (2015) reviewed Dog Rabies vaccination coverage in Africa based on published research articles on percentage of dogs owned and percentage of dogs vaccinated against rabies and on the relation between vaccination coverage and cost recovery. Review results indicated that only a small percentage of African dogs were ownerless. Puppies younger than 3 months of age constituted a considerable proportion of the African dog population. There were considerably more male dogs than female dogs present within the dog population. Reported that the dog rabies parenteral vaccination coverage following a “free of charge” vaccination scheme (68%) was closer to World Health Organization recommended threshold coverage rate (70%) compared to the coverage rate achieved in “owner-charged” dog rabies vaccination schemes (18%).

Bedekovic *et al.* (2016) in Europe evaluated use of ELISA for the detection of rabies virus antibodies from the thoracic liquid and muscle extract samples in the monitoring of fox oral vaccination campaigns. The specificity of the test for not-heat treated samples was 92.98 percent and sensitivity 79.20 percent. Diagnostic validity of the ELISA compared to the mFAVN test when not-heat treated samples were used was 89.16 percent . The specificity of the test for heat treated samples was 79.10 percent and sensitivity 96.36 percent. Reportedly, the diagnostic validity of the Bio Pro ELISA compared to the mFAVN test for heat treated samples was 94.30 percent.

### **2.3 QUANTITATION OF ANTI-RABIES ANTIBODIES IN DOG SERA**

Zalan *et al.* (1979) described RFFIT for quantitation of Rabies virus neutralizing antibodies. The new test, performed in the wells of microplates combined the advantages of the RFFIT with those arising from the use of a micro technique. Suggested that the described technique was as reproducible as the mouse neutralization test and accordingly recommended for the quantitative assay of rabies virus neutralizing antibodies.

Derbyshire and Mathews (1984) in Ontario studied the antibody titres against rabies in pet dogs. Rabies virus neutralizing antibody titres were determined by the microtest modification of the rapid fluorescent focus inhibition test before and after primary vaccination in 30 puppies, and before and after booster vaccination in 59 previously vaccinated dogs using a commercial modified live virus vaccine. Three weeks

after primary vaccination, the mean antibody titre reportedly was  $102 \pm 90$ , but only 24 dogs presented for booster vaccination had detectable antibody levels (mean titre  $12 \pm 16$ ). They found that the antibody responses three weeks after booster vaccination (mean  $380 \pm 216$ ) were significantly greater than the responses to primary vaccination.

Jayakumar *et al.* (1989) in Madras inoculated Mongrel dogs once daily for seven days with 2 ml of a vaccine prepared from 5 percent suspension of sheep brain inactivated with  $\beta$  propiolactone. Their humoral immune response was monitored by enzyme immune assay at intervals upto one year post vaccination. IgM was formed during the early immune response, which lasted for up to 6 weeks, but subsequently IgG became dominant. A local strain of rabies street virus was used for challenge infection by inoculation in the masseter muscle. They reported that all vaccinated dogs withstood challenge infection one year after vaccination, while all unvaccinated dogs died from rabies.

Morshedi and Aslani (2002) evaluated anti-rabies antibody titre in different time after vaccination and number of administered doses in pet dogs and compared with non-vaccinated and stray dogs in Urmia. Out of 60 sera from vaccinated dogs, 49 (81.6%) had protective antibody titres between 0.5 to 2.40  $\mu\text{l/ml}$ , but non-vaccinated pet dogs and stray dogs didn't show the valuable titre of Antibodies. Reportedly, the highest titre obtained in dogs was 2.40 $\mu\text{l/ml}$ , with frequency of six samples, which have received three doses of rabies vaccines.

Cliquet *et al.* (2003) in U.K. conducted a study on the development of a qualitative indirect ELISA for the measurement of rabies virus-specific antibodies from vaccinated dogs and cats. In the Study I, the number of false-positive and false-negative results as reported were 11(1.1%) and 67(6.7%) samples respectively. In the Study II, the number of false-positive and false-negative results reportedly were 7(0.8%) and 52 (5.7%) samples. In the Study III, 1 false-positive (0.23%) and 91 false-negative (20.7%) samples were identified. ELISA was reported to have a lower sensitivity than the FAVN test, but was considered as a useful tool for rapidly screening serum samples from vaccinated companion animals.

Fachin *et al.* (2005) in Brazil compared Serological Methods for Detecting the Immune Response after Rabies Vaccination in Dogs and Cows from Rabies-Endemic Areas. Post-vaccination titers measured by LPC-ELISA (Lysophosphatidylcholine ELISA) for vaccinated dogs and cows correlated closely with those found by FAVN ( $r = 0.893$ ). The LPC-ELISA presented relative specificity of 98.00 percent, sensitivity of 99.49 percent, and accuracy of 100 percent when the titers were compared with those obtained by FAVN for the dog sera. For the cow sera, the same accuracy was observed with the relative specificity of 86.90 percent and relative sensitivity being lower (98.43%). They found that LPC-ELISA using polyclonal immune reagents could be valuable for the detection of rabies antibodies, with low cost and reliable results during vaccination campaigns, especially in undeveloped countries.

Feyssalguet *et al.* (2006) carried a comparative study of new ELISA, Platelia rabies II, with RFFIT for detection and titration of anti-rabies glycoprotein antibodies in vaccinated and non-vaccinated people. A total of 1348 vaccinated or non-vaccinated people were tested in parallel using the new ELISA and the RFFIT for the presence of anti-rabies antibodies. The sensitivity reportedly reached 98.6 percent and the specificity 99.4 percent. The results found in their study reportedly indicate that the new ELISA test to be as sensitive and specific as the standardized reference method (VNT).

Servat *et al.* (2006) in France used ELISA (Platella rabies II Kit) to measure the level of rabies anti-glycoprotein G antibodies after vaccination in different populations of dogs, cats and foxes. Test was found to be highly specific irrespective of the species (more than 98%) using a cut-off value of 0.5 IU/ $\mu$ l. The false negative results ranged between 8.9 percent and 11.1 percent and the results of false positive results ranged from 1% and 2% for dogs and cats population, respectively.

Mughale *et al.* (2012) in Punjab carried a study to elucidate the serological response in 183(180 dogs and 3 large animals) suspected rabies exposed cases of small and large animals presented Veterinary Clinical Complex from July 2010 to June 2011. They assessed the Immune status by ELISA. In vaccinated dogs, rabies virus specific antibodies (0.5 IU/ $\mu$ l) were detected in 70.55 percent (115/163) cases. Whereas 29.44 percent vaccinated dogs did not reveal protective rabies titre. None of the unvaccinated

dogs and large animals showed protective rabies specific antibody titer. Reportedly, the antibody level was directly proportional to the number of vaccine shots. Vaccination in dewormed animals revealed better protection and antibody titre (8.878 IU/ $\mu$ l) compared to non-dewormed animals (4.01 IU/ $\mu$ l).

#### **2.4 ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) AMONGST DOG OWNERS REGARDING CANINE RABIES**

Kaliyaperumal (2004) in Madurai presented the general guidelines for conducting a Knowledge, Attitude and Practice Study. The main purpose of KAP study carried was to explore differences in Knowledge, Attitude and Practices of the community, paramedical personnel and medical practitioners about diabetes and diabetic retinopathy. The study provided information for valuation of the diabetic retinopathy programme. It revealed increase in knowledge, changes in attitudes towards diabetes and diabetic retinopathy as well as changes in the kinds of practices that were being followed regarding management of diabetes and diabetic retinopathy. They further observed that the properly collected and analyzed data, as presented in a report may be distributed to other interested parties.

Altmann *et al.* (2008) in France assessed the awareness about mode of rabies transmission, travel-associated rabies risk, and adequate preventive measures among French travelers using KAP study. Reportedly, only 6.7 percent of travelers under study knew that the risk of rabies was important, while 40.1 percent considered it moderate or low. Dog bites appeared to be a well-known mode of transmission of rabies. By contrast, licks on broken skin or contamination of the mucous membrane with saliva (10%) and scratches (0.7%) were least known.

Matibag *et al.* (2008) in Sri Lanka conducted pilot study on the usefulness of information and education campaign materials in enhancing the knowledge, attitude and practice on rabies in the rural areas. Respondents showed inclination toward animal birth control (60%) rather than elimination (killing of dogs) methods (24%). There was disparity between pet owners enthusiasm to take pets for rabies inoculation (57%) and their ability to show dog vaccination cards (40%). Reportedly, pet owners were twice

more than non-pet owners to believe that it was their responsibility to control the population of roaming pet dogs (OR=1.78; 95% CI: 1.14 to 2.76). The rabies leaflets were very effective in informing the people on how to deal with rabid animals, the earliest schedule for anti-rabies vaccine (ARV) and repeat vaccination.

Prakash *et al.* (2012) in Pune conducted community-based epidemiological study on KAP regarding rabies prevention and control. All study participants as reported knew about rabies transmission by dog bite compared to only 23 percent having knowledge about its transmission by scratches and licks. Participants (40%) knew that the disease being fatal, 55.5 percent of participants were aware about role of vaccine in preventing rabies.

Tenzin *et al.* (2012) in South-Central Bhutan conducted a community based study on knowledge, attitude and perception of rabies. Out of 615 household respondents interviewed, 224 (36%) were males and 391 (64%) were females. The majority of the respondents had high level of knowledge, and attitude and perception of rabies and had a positive attitude towards the prevention and control of rabies. The majority of the respondents believed that stray dogs were a problem in the community and felt that it was important to control the dog population in the Gelephu area. Reportedly, they found knowledge gap about rabies in the community that could be improved by awareness or education programme.

Ali *et al.* (2013) in Ethiopia assessed the knowledge, attitudes and practices of the communities on rabies. A cross-sectional study design and multistage sampling procedures were employed to select households for this study. Kebeles were randomly selected using lottery method from list of kebeles in each sub city, followed by selection of 42 households from each kebeles using systematic random sampling method. The data were collected from 1260 households through face to face interview using pre tested and structured questionnaires. The reports suggested that there was strong association between knowledge scores and sex; educational level; occupation and household size ( $p < 0.05$ ). However, statistically significant difference was observed only between attitude scores and age; educational level and monthly income ( $p < 0.05$ ). Study revealed that higher scores in practices to prevent rabies were recorded in male respondents those who completed higher education ( $p < 0.05$ ). Moreover, there was a strong

positive correlation between knowledge and practice; attitude and practice. A moderate level of awareness was shown with regard to rabies among the residents of Addis Ababa area according to the reports. Additionally, inaccessibility to appropriate services was also considered as a major reason for the low level of community participation in rabies prevention and control activities.

Sambo (2013) in Tanzania studied KAP related to rabies, its prevention and control. Participants who were knowledgeable about rabies had better behavioural practices compared to those who were less knowledgeable. Study highlighted the potential of communicating public health information to address knowledge gaps and improve rabies control and prevention.

Singh *et al.* (2013) in Haryana conducted a study about myths and false beliefs associated with wound management such as application of oils, herbs and red chillies on wound inflicted by rabid animals and not washing the wound properly. The aim of the study was to assess the knowledge and practices among the general practitioners (GPs) regarding dog bite management. The study population composed of 100 GPs comprising 45 MBBS or above degree holders (Group 1) and 55 other GPs like BAMS, RMPs, etc. (Group 2). Out of the total, 68 and 29 percent respondents in Group 1 and Group 2, respectively, correctly told that wound must be washed with soap and water for minimum period of 15min. A total 71 and 11 percent respondents in Group 1 and Group 2, respectively, could correctly answer about the target groups for pre-exposure prophylaxis. A total 62 percent GPs did not know the high risk groups to whom pre-exposure prophylaxis had to be given. Overall reports suggest that there was lack of awareness among the GPs regarding appropriate animal wound management and vaccine administration.

Kulkarni *et al.* (2013) in India conducted a pilot study to know about the perception of women self help group (SHG) members regarding rabies and its prevention in urban Mysore. Of the total 110 subjects enrolled in the study, majority 43 (39.1%) were in the age group of 35-44 years. Overall perception regarding rabies and its prevention was found to be poor in 59 (53.6%). Twenty-eight (25.5%) respondents were aware of disease rabies, seven (25.0%) replied that rabies was fatal and 31 (28.2%) replied that the dog/animal bite should be washed with soap and water. The overall

perception regarding rabies and its prevention amongst women self help group members was reported to be poor.

Guadu *et al.* (2014) in Ethiopia conducted a study to assess the KAP on rabies and associated factors. According to the reports, majority of study participants (64.1%) had good level of KAP. A strong association between KAP scores and sex ( $\chi^2=69.624$ ,  $p<0.05$ ); educational level ( $\chi^2 =1.893$ ,  $p<0.05$ ); occupation( $\chi^2 =58.554$ ,  $p<0.05$ ) and household size ( $\chi^2=38.069$ ,  $p<0.05$ ) was reported.

Veronica *et al.* (2014) in Nigeria designed a descriptive cross sectional study to assess KAP of dog owners towards rabies. Reportedly, dog owners (civil servants) possessed 4.8 times more likely to have good knowledge than those of other occupation groups. Positive attitude towards rabies reportedly increased with increase in age of dog owners. The respondents within the age group 20-30 years more likely had negative attitude than those over 40 years. Civil servants were 9.8times more likely had good practice than other occupation groups.

Valekar *et al.* (2014) in India conducted a cross-sectional study of awareness regarding dog bite and its management in rural community of Maharashtra. A total of 144 participants were included in the study, 75 (52%) were females and rest were males (48%). Reports suggest that out of these 111 (77%) knew that dog bite causes disease and amongst these 52/111 (46.8%) were aware that dog bite causes rabies. Among the participants, 138 (95.8%) were aware about the availability of vaccine and 125 (90.5%) knew that the vaccine was available in Government Hospital. In the study 88% of dog bite victims received treatment. A lack of awareness regarding dog bite and its management among the rural population was reported.

Kishore *et al.* (2015) conducted KAP on Health workers regarding Rabies and its Prevention in district Dehradun of Uttarakhand. Most of the study participants (59.9%) obtained a medium score (10-17) in knowledge regarding disease and its prevention. All of the participants had heard of the disease and knew about its mode of spread. Most of the participants (83.3%) lacked knowledge about anti-rabies immunoglobulin and pre-exposure prophylaxis (80.2%). The 81.4 percent participants had attitude scores in

satisfactory range (2-3). Sixty percent had practice scores in low range (0-2). Though the knowledge regarding pet vaccination and full course of human vaccination was lacking in some but the willingness to encourage pet owners to vaccinate their pets and encourage bite victims to get full course of vaccine was 100 percent. Reportedly, they suggested that there was a need for sensitization of health workers so that their knowledge can be enhanced and their positive attitude (100% willingness) can be translated into proper practices for prevention and control of Rabies.

Tondare *et al.* (2015) in India assessed the effectiveness of structured teaching programme on knowledge among mothers of school age children regarding first aid management of dog bites in selected areas of Raichur, Karnataka with sample size of 30. Evaluative approach with one group pre test and post test pre-experimental design was selected to achieve the objectives of the study. Reports revealed that the mothers of school age children responded inadequately. Majority of mothers of school age children got adequate knowledge after structured teaching programme. Significant difference was found in pre test and post test scores of the mothers. In pre test it was observed that there was not even a single mother who possessed high knowledge. Whereas in the post test, mothers possessed high knowledge about first aid management of dog bite.

Kamble *et al.* (2016) presented a study on KAP related to animal bites among the residents of an Urbanised Village in South Delhi. Animal bites were amongst the major public health issues, not only for the risk of acquiring secondary infections but also for the possibility of contracting rabies. It was a cross-sectional study carried out on 330 adult participants (M : F ratio 0.61:1). Face to face interview was conducted using semi-structured, pre-tested questionnaire after taking a written informed consent. Reportedly, around 75 percent of the respondents reportedly had correct knowledge regarding the consequences of animal bites. Nearly 94.5 percent of the respondents consulted doctors and 3.3 percent respondents had faith in traditional healers and jhaad phook. Reports also revealed that 40.3 percent of the respondents consider the application of indigenous substances on animal bite wounds like chillies, turmeric, and herbal paste useful.

Joice *et al.* (2016) presented study on Knowledge, Attitude and Practices regarding dog bite and its management among adults in rural Tamil Nadu and to evaluate

association between selected socio-demographic factors. Community based cross sectional study was conducted on 275 households, of which only 6.9 percent knew microorganism as the cause of rabies but 86.2 percent of the respondents knew rabies was spread by dogs. Study revealed 90.5 percent and 59.3 percent knew that a vaccine for rabies is available and the site of administration as abdomen respectively. Reports also found that 73.8 percent felt it was essential to wash the bite site with soap and water. Only 49.4 percent practiced deep burial as the method of disposal of a carcass of suspected rabid dog. The knowledge, attitude and practices with respect to prevention and treatment of rabies were not found adequate among the rural population as per the study.

Chapter-III

Materials  
and  
Methods

**3.1 PLACE OF WORK**

The research work was carried out in the Division of Veterinary Public Health and Epidemiology, Faculty of Veterinary Sciences and Animal Husbandary, SKUAST-J, R.S. Pura, Jammu. The period of study was from June, 2016 to April, 2017.

**3.2 EVALUATION OF IMMUNE RESPONSE TO RABIES VACCINATION IN PET DOGS**

A total of 180 sera samples were collected aseptically of various categories (**Category I –First time vaccination, Category II – First booster and Category III – Yearly booster**) of vaccinated dogs from in and around Jammu region. The distribution of collected samples as per area and category of dogs as per vaccination schedule has been shown in Table 1. The sampling in animals was done in a randomized manner, with a history of vaccination; whether brought for first time vaccination, first booster or yearly booster. Moreover, information on age and sex wise categorization done in particular group of dogs is as presented in Table 2 and 3 respectively. All the samples were subjected to ELISA tests (qualitative as well as quantitative).

**3.2.1 Determination of Immune Response to Anti-rabies vaccine**

In order to determine the immune response of the anti-rabies vaccine among dogs, the blood samples were collected from veterinary clinical cases and CVH, Talab Tillo and private clinics, the details of which are described in the text that follows. The results are compared with the standards recommended by the test kit.

**3.2.2 Collection of Serum Samples**

For serum collection, the blood from dogs was withdrawn aseptically from cephalic or sephanous vein puncture in disposable syringes (5 ml), shifted to vacutainers and kept in slanting position, allowed to clot for 2 to 4 hours at room temperature. The serum was separated by centrifuging the vacutainers at 3000 rpm (at 4 °C) for 7 minutes.

The collected serum samples were duly marked and aliquoted before being stored at  $-20^{\circ}\text{C}$  until used further as recommended by WHO.

**Table 1: Details of serum samples collected from dogs as per area and age group in and around Jammu region**

PLACE OF SAMPLE COLLECTION	Category of dogs as per Vaccination Schedule		
	Category I	Category II	Category III
CVH, Department of Animal Husbandry, Talab Tillo , Jammu (Government of J&K)	17	48	89
Veterinary Clinical Complex, SKUAST-J	2	3	6
Private clinics in Jammu	2	7	6
TOTAL (180)	21	58	101

The sample rate is higher in the Category III (n=101) followed by Category II (n=58) and Category I (n=21).

**Table 2: Age-wise distribution of dog sera samples**

<b>Category (no.)</b>	<b>Age group</b>	<b>No. of samples</b>
<b>Category I (21)</b>	<3 months	5
	3 months	7
	>3 months	9
<b>Category II (58)</b>	4-6 months	38
	7-9 months	13
	10-12 months	6
	>1 year	1
<b>Category III (101)</b>	1.5-3 years	65
	3.5-5 years	18
	5.5-7 years	6
	7.5-above	12
<b>Total</b>		<b>180</b>

**Table 3: Sex-wise distribution of dogs sera samples**

<b>Species (No. of samples)</b>	<b>Sex</b>	<b>No. of samples</b>
<b>Dogs (180)</b>	Male	131
	Female	49

### 3.2.3 Equipments

In the present study equipments used were- ELISA reader (Bio-Rad Model 680), Centrifuges (R8C Laboratory Centrifuge, REMI; Sorvall RC – 5B refrigerated super speed centrifuge).

### 3.2.4 Chemicals and Reagents used

All the chemicals used were of analytical grade in present study.

### 3.2.5 Buffers and Reagents

Detailed composition of various buffers and reagents (supplied in the kit) used in the present study are given below:

#### 3.2.5.1 Contents of ELISA kit

<b>Microtitre strips</b>	<b>12×8</b>
<b>Strip holder</b>	<b>1</b>
<b>ELISA buffer (green cap)</b>	<b>1×18 ml</b>
<b>HRPO conjugated anti-species antibodies (ready to use</b>	<b>1×12 ml</b>
<b>Positive control</b>	<b>1×0.5 ml</b>
<b>Negative control</b>	<b>1×1.0 ml</b>
<b>Wash-solution (200x concentrated)</b>	<b>1×20 ml</b>
<b>Substrate A</b>	<b>1× 8 ml</b>
<b>Substrate B</b>	<b>1× 8 ml</b>
<b>Stop-solution</b>	<b>1× 8 ml</b>
<b>Plastic cover</b>	<b>1</b>

### 3.2.6 Preparation of Indirect Enzyme linked immunosorbant assay (ELISA)

The I-ELISA was performed as per the method standardized by the B.V. European Veterinary Laboratory(manufacturers). The diagnostic test-system for the establishment of Rabies infection was intended to identify antibodies against epitopes of rabies virus, in serum samples. In contrast to other test systems this standardized ELISA was based on whole-inactivated virus, reportedly (as per manufacturers) had a very high sensitivity and specificity.

### **3.2.6.1 Principle of the test**

The test is based on the reaction of semi purified virus with polyclonal dog antibodies. To 96 wells microtitre plates precoated with synthetic peptides were used in the tests.

### **3.2.6.2 Preparation and dilution of the sera samples**

#### **3.2.6.2.1 Qualitative ELISA**

The dog sera samples were diluted in 1:250 ratio and added to the wells of the pre coated plate as per the format of test.

#### **3.2.6.2.2 Quantitative ELISA**

The dog serum sample for carrying out quantitative ELISA were titrated using a 3-times dilution, starting with a dilution of 1:50 followed by  $\rightarrow 1:150 \rightarrow 1:450 \rightarrow 1:1350$ . The plates were washed using the wash buffer by executing a cycle of 5 times.

After washing, the bound dog antibodies were detected by using HRPO conjugated anti-species conjugate. The color reaction in the wells was directly related to the concentration of rabies virus antibodies in the serum sample.

### **3.2.6.3 Wash Protocol**

In ELISA's, uncomplexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter and intra assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. In the presented study was has been performed manually which involves following steps:

1. The microtitre plate was emptied by turning it upside down, followed by a firm vertical downward movement to remove the buffer.
2. All the wells were filled with 250  $\mu$ l washing solution prepared.
3. This washing cycle was carried out atleast 5 times.

4. Plate was turned upside down and emptied with a firm vertical movement.
5. Inverted plate was then placed on absorbent paper towels and the plate then tapped firmly to remove any residual washing solution in the wells.

#### **3.2.6.4 Performing ELISA Test**

While preparation for test, the reagents were taken out of the kit and placed at room temperature for  $\pm 15$  minutes without exposing them to direct sunlight, light or heat sources. As per instructions buffer, controls, standards and conjugates were shaken gently before use, in order to dissolve/mix any component that may have been precipitated. Vials were gently tapped against the table, in order to pull back any fluid that was still retained on the cap. The reagents were stored back at 4-8 °C immediately after use.

#### **3.2.6.5 Test Protocol Qualitative**

After opening the packet of stripes, they were washed 5 times with washing solution according to the prescribed wash protocol. The washing solution provided was diluted from 200X to 1X by taking 995 ml of aquabidest water and 5 ml of 200X wash solution and the pH of buffer was checked to be neutral by pH meter.

The positive and negative controls were reconstituted before use by 0.5ml and 1.0ml of aquabidest water respectively. The dilutions (1:50) were prepared for positive and negative controls in round bottomed plate by taking 122.5 $\mu$ l of ELISA buffer and 2.5 $\mu$ l of both the controls in the respective wells. Subsequently, the dilutions (1:250) of 90 serum samples were prepared in their respective wells by taking 124.5 $\mu$ l of ELISA buffer and 0.5 $\mu$ l of the serum sample. After the constitution of dilutions, 100 $\mu$ l of each one of the dilutions including all the sera samples along with both the controls (positive and negative) were transferred to the wells of ELISA plate. In the remaining wells of the ELISA plate, ELISA buffer @100 $\mu$ l were placed as substrate control . The plate was sealed and incubated for 60 min. at 37 °C. The plate was subjected to 5X washing as per the wash protocol. Following which each well was dispensed with 100  $\mu$ l of conjugated anti-species antibody, sealed and incubated for 60 min. at 37 °C. After incubation, washed (5 X), and Buffers A and B (gently mixed) were added @ 100  $\mu$ l per well. Followed by incubation for 10-20 minutes in the dark at room temperature (21 °C). The stop solution

@50  $\mu$ l was added to each well and absorption values recorded using ELISA reader within 10 minutes at 450 nm. The substrate control also served as blank.

### **3.2.6.6 Test Protocol Quantitative**

After opening the packet of stripes, they were washed 5 times with washing solution according to the prescribed wash protocol. The washing solution provided was diluted from 200X to 1X by taking 995 ml of aquabidest water and 5 ml of 200X wash solution and the pH of buffer was checked to be neutral by ph meter.

The positive and negative controls were reconstituted before use by 0.5ml and 1.0ml of aquabidest water resp. The 3 step dilutions (1:50→1:150→1:450→1:1350) were prepared for positive and negative controls in round bottomed plate by taking 122.5 $\mu$ l of ELISA buffer in 4 wells of round bottomed plate and 2.5  $\mu$ l of both the controls in their respective wells and making serial dilution afterwards. Subsequently, the dilutions (1:50→1:150→1:450→1:1350) of the 90 serum samples were made in their respective wells by taking 122.5 $\mu$ l of ELISA buffer and 2.5  $\mu$ l of the serum sample and making serial dilution afterwards. After the constitution of dilutions, 100 $\mu$ l of each one of the dilutions including all the sera samples along with both the controls (positive and negative) were transferred to the wells of ELISA plate. In the remaining wells of the ELISA plate, ELISA buffer @100 $\mu$ l were placed as substrate control. The plate was sealed and incubated for 60 min. at 37°C. The plate was subjected to 5X washing as per the wash protocol. Following which each well was dispensed with 100  $\mu$ l of conjugated anti-species antibody, sealed and incubated for 60 min. at 37°C. After incubation, washed (5 X), and Buffers A and B (gently mixed) were added @ 100  $\mu$ l per well. Followed by incubation for 10-20 minutes in the dark at room temperature (21°C). The stop solution @50  $\mu$ l was added to each well and absorption values recorded using ELISA reader within 10 minutes at 450 nm. The substrate control also served as blank.

### 3.2.6.7 Validation of the tests

#### Qualitative I-ELISA:

The tests were validated to meet the following criteria as per the manufacturers protocol. The mean value (MV) of the measured OD value of the Positive Control (PC) must be  $\geq 1.000$ . The MV of the measured OD value for the Negative Control (NC) must be  $\leq 0.400$ .

#### Quantitative I-ELISA:

To confirm appropriate test conditions the OD of the positive control should be  $\geq 1.000$  OD units (450 nm) and given end point titre of  $\geq 150$ . The negative control should be  $\leq 0.400$  OD units (450 nm) and give an end point titre of  $\leq 50$ .

### 3.2.6.7 Calculation:

**Qualitative:** The mean value (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC) were calculated.

The ratio (S/P) of sample OD to mean OD of the positive control was calculated according to the following equation (as per kit protocol):

$$S/P = \frac{OD_{\text{sample}} - MV_{\text{OD of NC}}}{MV_{\text{OD-PC}} - MV_{\text{OD of NC}}}$$

Where S/P=Samples/Positive, MV=Mean values, OD=optical density, PC=positive control, NC= negative control

**Quantitative:** The ELISA titre were calculated by constructing a curve and using cut-off line (dilution 1:50-1:150-1:450 and 1:1350) with OD on Y-axis and titres on X-axis. ELISA titres were calculated using a cut-off 2.5 times the OD value of negative control at 1:50.

The Fluorescent Antibody Virus Neutralisation (FAVN) titre of the positive control was 1.83 IU (as per the manufacturers). The K-factor was calculated by dividing the obtained ELISA positive titre by 1.83 to get K factor in IU.

All the ELISA titres obtained in the constructed graph were divided by K factor so as to obtain FAVN titres in IU.

### **3.3 ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) OF DOG OWNERS TO CANINE RABIES IN REGION OF JAMMU**

#### **3.3.1 Study area and sample size**

The survey was conducted in Jammu district, area located in South-Western part of state Jammu and Kashmir. The respondents in the study were grouped as per the dwellers area of living viz; urban and rural. A total of 200 respondents were interviewed with 130 participants (65 %) from urban region and 70 participants (35%) from rural areas.

The participation was voluntary and data collection was kept confidential to meet the ethical considerations. The questionnaire based interview was conducted in order to assess the knowledge, attitude and perceptions of rabies.

#### **3.3.2 Questionnaire design**

A questionnaire designed for this study was partly adapted from similar such studies conducted elsewhere (Matibag et al., 2007; Matibag et al., 2009; Bingham et al., 2010) and consisted of few closed and open questions. The designed questionnaire to obtain information comprised of 4 parts: questions pertaining to the respondent's socio-demographic information (age, sex, education level, occupation, religion, ethnicity, number of people in the household, dog ownership status, location); questions related to the knowledge and perception of rabies; questions related to attitudes and perception of rabies and its control activities; and questions on pet care practices (asked only to dog owners). The questionnaire was piloted on limited scale on the people having nearly similar background before the actual survey in order to improve clarity and interpretation. The final structured and used Questionnaire is as follows :-

**Survey Proforma on Knowledge, Attitude and Practice (KAP)**

Questionnaire No:

Date:

**Part A: General Information:**

- 1. Informant's name and address :**
- 2. Contact Details :**
- 3. Age :**
- 4. Sex:**
- 5. Education status:**
  - a. Illeterate
  - b. Primary
  - c. Middle
  - d. High school
  - e. Diploma
  - f. Graduate/above
- 6. Occupation:**
  - a. Student
  - b. Farmer
  - c. Employee
  - d. Business
  - e. Dependent/Housewife
  - f. Unemployed
- 7. Religion:**
  - a. Hindu
  - b. Muslim
  - c. Christian
  - d. Others
- 8. Location:**
  - a. Urban
  - b. Semi urban
  - c. Rural
- 9. No. of family members:**
  - a.  $\leq 5$
  - b.  $> 5$
- 10. Dog ownership status:**
  - a. Yes
  - b. No

**Part B: Community Knowledge and perception of Rabies:**

- 1. Have heard of rabies?**
  - a. Yes
  - b. No
- 2. Believe that rabies is a fatal disease?**
  - a. Yes
  - b. No
- 3. Believe that rabies can be transmitted by dogs?**
  - a. Yes
  - b. No

- 4. If dog owned, at what age should it receive 1<sup>st</sup> rabies vaccine?**
  - a. Yes
  - b. No
- 5. Believe that bite wound should be washed with soap and water?**
  - a. Yes
  - b. No
- 6. Believe that rabies can be prevented by vaccination of dogs?**
  - a. Yes
  - b. No
- 7. Believe that suspected rabies can be confirmed by laboratory tests?**
  - a. Yes
  - b. No
- 8. Believe that there are no locally available methods of treatment for dog bite and rabies?**
  - a. Yes
  - b. No

**Part C: Community Attitudes and perception of Rabies:**

- 1. Would report to hospital for treatment if bitten by a stray dog?**
  - a. Yes
  - b. No
- 2. Would report to hospital for treatment if bitten by owned dog?**
  - a. Yes
  - b. No
- 3. Would report to authorities if there is suspected outbreak of rabies in the community?**
  - a. Yes
  - b. No
- 4. Would kill stray dog if rabies is suspected?**
  - a. Yes
  - b. No
- 5. Is stray dog a problem in your community?**
  - a. Yes
  - b. No
- 6. Believe it is important to control dog population in Jammu?**
  - a. Yes
  - b. No
- 7. Do you support rabies control campaign?**
  - a. Yes
  - b. No

#### **Part D: Community Practices and perception of Rabies:**

- 1. Believe it is good to vaccinate your dog?**
  - a. Yes
  - b. No
- 2. Dog handlers should wear protective clothing?**
  - a. Yes
  - b. No
- 3. It is good to wash dog bite wounds with soap?**
  - a. Yes
  - b. No
- 4. Dog handlers should take human anti-rabies vaccine ?**
  - a. Yes
  - b. No
- 5. Have you provided shelter to your pet?**
  - a. Yes
  - b. No
- 6. Do you deworm your pet dog?**
  - a. Yes
  - b. No

#### **3.4. Sampling procedure**

The visitors who came to the various clinics including both governmental and non-governmental clinical complexes were interviewed. The volunteers included in the study were in the age group of 18 to 65 years. Face to face interview was conducted on the selected persons who were pre-informed about the purpose of the study. The interview was carried out between September to mid December. Answers were recorded in English.

#### **3.5 Data management and analysis**

Data were entered, managed and the analysis was carried using SPSS software v.16 and Microsoft Excel 2007.

#### **3.6 Descriptive Statistics**

Descriptive statistics were calculated for each variable of interest. General Linear Model (MANOVA) and Bivariate  $\chi^2$  test analysis were applied to compare the responses of the questions related to socio-demographic characters of respondents with respects to the respondent's knowledge, attitude and perception of rabies and difference between respondents from urban and rural areas as well as dog owners and non-dog owners respectively. A p-value <0.05 was considered statistically significant.

## Chapter-IV

# Results

Rabies is a fatal but vaccine preventable disease which is regarded as one of the most important zoonotic disease in India. Dogs are the most important source of transmission of rabies in the country including J&K. Therefore, the present study was undertaken to evaluate the immunological response in dogs against anti-rabies vaccination and Knowledge, Attitude and Practice assessment of the dog owners. No such study seems to have been carried out in Jammu so as to generate data on the vaccination response in order to test the immune status of population. A total of 180 serum samples were taken which were classified into three main categories as per vaccination of dogs viz., Category I (First time brought for vaccination), Category II (First booster) and Category III (Yearly booster). The sera samples were collected from dogs brought for vaccination to Veterinary clinical complex, Department of animal husbandry, Govt. of J&K and private clinics. A questionnaire based survey was also conducted among the owners of dogs which were categorized on the basis of dog ownership status and their location (rural/urban). The results obtained are presented in this chapter.

### **4.1 EVALUATION OF IMMUNE RESPONSE TO RABIES VACCINATION IN PET DOGS**

The dogs sera samples (180) were collected from different sources comprising 154 samples from CVH, Department of Animal Husbandry, Talab Tillo, Jammu, 11 samples from Veterinary Clinical Complex, SKUAST-J and 15 samples from private clinics from Jammu. The details of samples collected are presented in Table 4. Analysis of 180 samples of vaccinated dogs was carried out by Indirect ELISA and the overall sero-prevalence both qualitatively and quantitatively was assayed. Results are presented in Table 5 and 6.

**Table 4: Sero-prevalence of anti-rabies antibodies in sera samples of dogs (n=180) from different collection points as detected by Indirect ELISA**

<b>Place of Collection</b>	<b>No. of sample</b>	<b>Positive (%)</b>	<b>Negative (%)</b>
CVH, Department of Animal Husbandry, Talab Tillo, Jammu (Government of J&K)	154	75 (48.7)	79 (51.2)
Teaching Veterinary Clinical Complex, SKUAST-J	11	6 (54.5)	5 (45.4)
Private clinics in Jammu	15	8 (53.3)	7 (46.6)
<b>TOTAL</b>	<b>180</b>	<b>89</b>	<b>91</b>

\* Figures in the parentheses indicate the percentage value

A total of 180 samples from diverse clinical settings were collected with maximum number being from CVH followed by Teaching Veterinary Clinical complex and Private clinics in Jammu. The sero-prevalence of anti-rabies antibodies as revealed by qualitative Indirect ELISA were CVH (48.7%), TVCC (54.5%) and Private Clinics (53.4%). The samples testing negative by Qualitative I – ELISA for anti-rabies antibodies were CVH (51.2%), TVCC (45.4%) and Private Clinics (46.6%).

The category wise details on ser-prevalence of anti-rabies antibodies in dog sera-samples (qualitatively and quantitatively) are presented in Table 5 and 6 respectively.

**Table 5: Sero-prevalence of rabies anti-bodies in dog sera samples assayed qualitatively by Indirect ELISA**

<b>Category (no.)</b>	<b>Age group</b>	<b>No.</b>	<b>Positive (%)</b>	<b>Negative (%)</b>
<b>Category I (21)</b>	<3 months	5	2	3
	3 months	7	2	5
	>3 months	9	4	5
			(38.09%)	(61.90%)
<b>Category II (58)</b>	4-6 months	38	15	23
	7-9months	13	3	10
	10-12months	6	4	2
	>1yr	1	1	0
			(39.65%)	(60.34%)
<b>Category III (101)</b>	1.5-3 years	65	36	29
	3.5-5 years	18	10	8
	5.5-7 years	6	3	3
	7.5-above	12	8	4
			(56.43%)	(43.56%)
<b>Total (180)</b>			<b>89 (49.44%)</b>	<b>91 (50.55%)</b>

\* Figures in the parentheses indicate the percentage value

The samples were categorized into three broad categories with positive results from Category I -39.09 percent, Category II -39.65 percent and Category III - 56.43 percent. From the above results, the sera samples from Category III seems to have higher anti-rabies antibodies assayed by Indirect ELISA.

**Table 6: Sero-prevalence of rabies anti-bodies in dog sera samples assayed quantitatively by Indirect ELISA**

<b>Category</b>	<b>Age Group</b>	<b>No.</b>	<b>Positive (%)</b>	<b>Negative (%)</b>
<b>Category I</b>	Upto 3 months	5	3 (60)	2 (40)
<b>Category II</b>	4 months – 1 yr	12	5 (41.66)	7 (58.33)
<b>Category III</b>	1 year and above	26	16 (61.53)	10 (38.46)
<b>Total</b>		<b>43</b>	<b>24</b> <b>(55.81)</b>	<b>19</b> <b>(44.18)</b>

\* Figures in the parentheses indicate the percentage value

Quantitative results were recorded as per the manufacturers protocol according to which titre of each sample was evaluated at 1:150 dilution by comparing value with negative control values at 1:50 dilution multiplied by a factor of 2.5 which was about 0.6 in experiment III and 0.66 in experiment IV.

#### **4.1.1 Sero-prevalence of anti-rabies antibodies after vaccination in dogs**

Further the analysis of the serum was carried out for different categories of vaccinated dogs, viz., age at vaccination, Categories of vaccination schedule (Category I - First time brought for vaccination, Category II - First booster and Category III - Yearly vaccination).

#### **4.2 EPIDEMIOLOGICAL PATTERN OF ANTI-RABIES VACCINATION**

The epidemiological pattern of anti-rabies vaccination was estimated for dogs for which age and sex were available. Accordingly inferences were made on the basis of results as depicted in Tables 7 and 8 with respect to age and sex respectively.

### 4.2.1 Age

Based on the age, the dogs were divided into 3 age groups i.e., 0->3 months, 4->1 year and 1.5 years and above. The category wise prevalence was recorded as 38.09 percent, 39.65 percent and 56.43 percent by ELISA, in the age groups 0->3 months, 4->1 year and 1.5 years and above respectively (Table7).

**Table 7: Age-wise sero-prevalence of anti-rabies vaccination**

<b>Age- group</b>	<b>No. of animals</b>	<b>ELISA POSITIVE (%)</b>
0->3 months	21	8 (38.09)
4->1 year	58	23 (39.65)
1.5 and above	101	24 (56.43)
<b>Total</b>	<b>180</b>	<b>89 (49.44)</b>

\* Figures in the parentheses indicate the percentage value

As per the table, the sero-prevalence on the basis of age was observed to be highest in the age group of 1.5 years and above.

### 4.2.2 Sex

Out of 180 sera samples collected, a total of 131 sera samples were from male dogs and 49 were from the females. The prevalence of anti-rabies antibodies was higher in males (50.38%) than in females (46.93%). (Table 8)

**Table 8: Details of sex-wise sero-prevalence of anti-rabies antibodies in vaccinated dogs**

<b>No. of samples</b>	<b>Sex (No. of samples)</b>	<b>ELISA Positive (%)</b>
Dog (180)	Males (131)	66 (50.38)
	Females (49)	23 (46.93)

\* Figures in the parentheses indicate the percentage value

### **4.3 DEMONSTRATION OF ANTI-RABIES ANTIBODIES BY QUALITATIVE ANALYSIS OF DOG SERA SAMPLES BY I - ELISA**

Demonstration of rabies antibodies (qualitative) in vaccinated dog sera samples was carried out by IgG based ELISA which represented the specificity i.e., either presence or absence of the anti-rabies antibodies in dogs sera samples. The test (experiment I and II) were performed for all the 180 sera samples (90 samples for each kit) reveals the status of anti-rabies antibodies in dog sera after vaccination and further evaluate the protection level. Positive results were obtained from 89 samples out of the total 180 sera samples. The details of optical density are presented in Table 9 and 10.

#### **4.3.1 Quantification based on cut-off value**

Cut-off value was calculated as per the manufacturer protocol. The positive and negative results of the sera samples were evaluated based on the cut-off value which was calculated 0.535 in Experiment I and 0.829 in Experiment II. The details of the evaluated results based on the Cut-off value were presented in the Table 9 and 10.

**Table 9: Results of Qualitative I-ELISA analysis of dogs sera samples in experiment I**

S.No	SAMPLE CODE	O.D	RESULTS	S.No.	SAMPLE CODE	O.D	RESULTS
1	13	0.302	Negative	46	1	0.884	Positive
2	14	0.6	Positive	47	2	0.65	Positive
3	42	0.549	Positive	48	4	0.396	Negative
4	56	0.372	Negative	49	5(a)	0.584	Positive
5	63	0.364	Negative	50	5(b)	0.71	Positive
6	78	0.44	Negative	51	15	0.518	Negative
7	82	0.829	Positive	52	10	0.583	Positive
8	89	0.3	Negative	53	16	0.701	Positive
9	95	0.468	Negative	54	17	0.861	Positive
10	110	0.267	Negative	55	19	0.597	Positive
11	122	0.534	Positive	56	20	0.332	Negative
12	129	0.432	Negative	57	21	0.397	Negative
13	140	0.272	Negative	58	23	1.004	Positive
14	142	0.675	Positive	59	26	0.38	Negative
15	168	0.359	Negative	60	28	0.765	Positive
16	3	0.505	Negative	61	30	0.75	Positive
17	6	0.498	Negative	62	38	2.956	Positive
18	12	0.406	Negative	63	39	1.996	Positive
19	22	0.455	Negative	64	43	0.401	Negative
20	25	0.499	Negative	65	46	1.108	Positive
21	32	0.503	Negative	66	47	0.673	Positive
22	34	0.312	Negative	67	48	0.465	Negative
23	37	0.51	Negative	68	67	0.655	Positive
24	49	0.508	Negative	69	68	0.498	Negative
25	50	0.268	Negative	70	53	0.422	Negative
26	55	0.316	Negative	71	60	0.689	Positive
27	59	0.98	Positive	72	70	0.74	Positive
28	72	0.381	Negative	73	74	0.514	Negative

29	84	0.617	Positive	74	80	1.466	Positive
30	87	0.943	Positive	75	86	1.087	Positive
31	92	0.486	Negative	76	90	1.02	Positive
32	96	0.681	Positive	77	91	0.691	Positive
33	98	0.908	Positive	78	103	0.598	Positive
34	104	0.465	Negative	79	107	0.474	Negative
35	113	0.613	Positive	80	111	1.643	Positive
36	116	0.416	Negative	81	115	0.491	Negative
37	118	1.052	Positive	82	120	0.535	Positive
38	119	0.64	Positive	83	126	0.587	Positive
39	149	0.757	Positive	84	144	0.7	Positive
40	156	0.363	Negative	85	147	0.538	Positive
41	160	2.531	Positive	86	151	0.305	Negative
42	143	0.773	Positive	87	152	0.887	Positive
43	161	2.012	Positive	88	166	0.269	Negative
44	124	0.422	Negative	89	172	0.474	Negative
45	101	0.312	Negative	90	173	1.08	Positive
Mean Positive O.D				1.00	Mean Negative O.D		0.224

S/P For +ve -  $\geq 0.4$ For - ve -  $< 0.4$ 

Cut off value (O.D) - 0.535

**Table 10: Results of Quantitative I-ELISA analysis of dogs sera in experiment II**

S.No.	SAMPLE CODE	O.D	RESULTS	S.No.	SAMPLE CODE	O.D	RESULTS
1	109	1.3010	Positive	46	51	0.884	Positive
2	128	1.5185	Positive	47	54	0.654	Negative
3	135	1.6990	Positive	48	58	0.765	Negative
4	2 (tvcc)	0.4330	Negative	49	64	0.3802	Negative
5	11	1.8195	Positive	50	69	1.6128	Positive
6	18	0.488	Negative	51	71	1.6990	Positive
7	27	0.829	Positive	52	73	1.6881	Positive
8	31	2.0414	Positive	53	75	0.5921	Negative
9	33	0.645	Negative	54	76	0.9937	Positive
10	36	2.1461	Positive	55	77	0.587	Negative
11	41	0.2430	Negative	56	81	0.332	Negative
12	52	0.1239	Negative	57	83	0.397	Negative
13	57	0.6021	Negative	58	94	0.267	Negative
14	61	0.6902	Negative	59	102	1.381	Positive
15	62	0.7482	Negative	60	105	2.093	Positive
16	85	0.5543	Negative	61	112	1.8573	Positive
17	88	1.8976	Positive	62	114	0.9638	Positive
18	93	0.302	Negative	63	65	0.0792	Negative
19	97	1.347	Positive	64	66	0.4521	Negative
20	99	0.499	Negative	65	121	0.612	Negative
21	100	1.9868	Positive	66	123	2.1584	Positive
22	101	2.0414	Positive	67	127	2.142	Positive
23	108	1.5643	Positive	68	136	0.655	Negative
24	117	1.6812	Positive	69	138	1.3575	Positive
25	125	1.0792	Positive	70	139	0.3483	Negative
26	130	0.1761	Negative	71	133	0.689	Negative
27	132	0.3424	Negative	72	134	2.3766	Positive
28	150	0.5011	Negative	73	141	0.7559	Negative
29	158	0.636	Negative	74	148	0.9031	Positive
30	175	1.6532	Positive	75	153	0.9912	Positive

31	176	0.2730	Negative	76	154	0.6481	Negative
32	6 (tvcc)	0.3108	Negative	77	155	0.452	Negative
33	7 (tvcc)	0.4771	Negative	78	157	0.598	Negative
34	8 (tvcc)	1.0512	Positive	79	159	0.474	Negative
35	8	1.1761	Positive	80	162	1.1271	Positive
36	9	0.386	Negative	81	163	2.1614	Positive
37	20	2.0414	Positive	82	164	0.535	Negative
38	21	1.0864	Positive	83	165	2.2201	Positive
39	24	2.1106	Positive	84	167	0.2201	Negative
40	29	2.1761	Positive	85	169	0.7243	Negative
41	32	1.0621	Positive	86	170	0.8573	Positive
42	35	2.1875	Positive	87	174	0.831	Positive
43	40	1.105	Positive	88	1 (tvcc)	0.351	Negative
44	44	2.230	Positive	89	4 (tvcc)	0.5012	Negative
45	45	0.312	Negative	90	5	2.1492	Positive
Mean Positive O.D			1.4255	Mean Negative OD			0.333

S/P For +ve -  $\geq 0.4$  For - ve -  $< 0.4$  Cut off value (O.D) – 0.829

#### 4.4 QUANTITATIVE ANALYSIS OF DOGS SERA BY I-ELISA

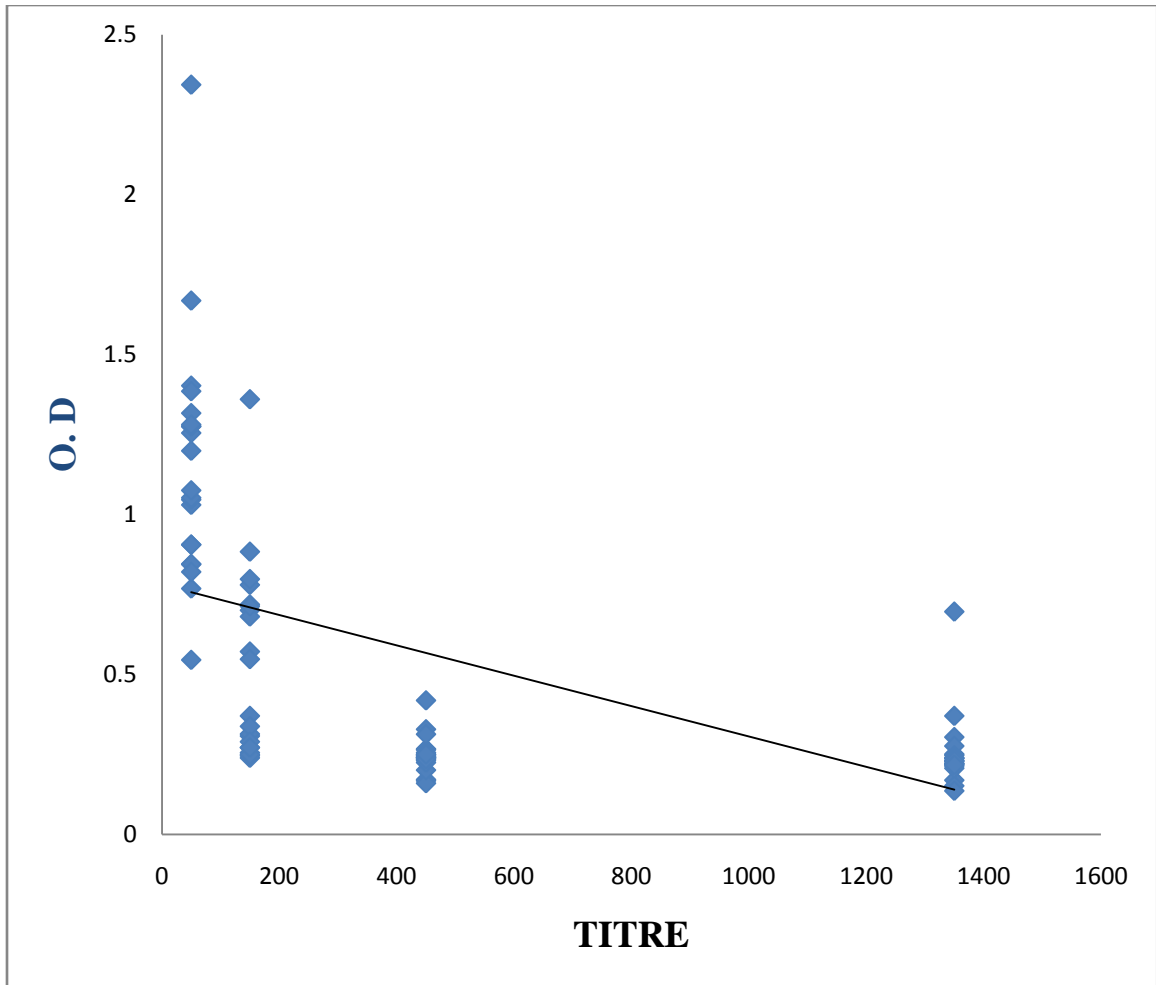
After qualitatively assaying the presence/absence of anti-rabies antibodies, the selected positive samples (43) were further assayed quantitatively to evaluate the presence of protective antibodies. The assays were carried out as per the protocol supplied by the manufacturers. Accordingly 43 positive samples were selected out of which 35 samples revealed protective titre above 0.5 IU/ $\mu$ l as calculated on the basis of protocol of kit. The detailed results (based on two experiments) are presented in the figure Fig. 1 to 5 (experiment III) and Fig. 6 to 10 (experiment IV).

The fig. 1 and 6 depicts the various titres of samples at dilutions of 1:50, 1:150, 1:450 and 1:1350. The titre kept on decreasing linearly as the dilution rate increased. The fig. 2 and 7 depicted the titre of positive samples at dilution of 1:50 in plate 1 and plate 2 respectively. The fig. 3 and 8 depicts the titre of the positive samples at dilution of 1:150

in plate 1 and 2 resp. The fig. 4 and 9 depicts the titre of the positive samples at dilution of 1:450 in plate 1 and plate 2 resp. The fig. 5 and 10 depicts the titre of positive samples at dilution of 1:1350 in plate 1 and plate 2 resp.

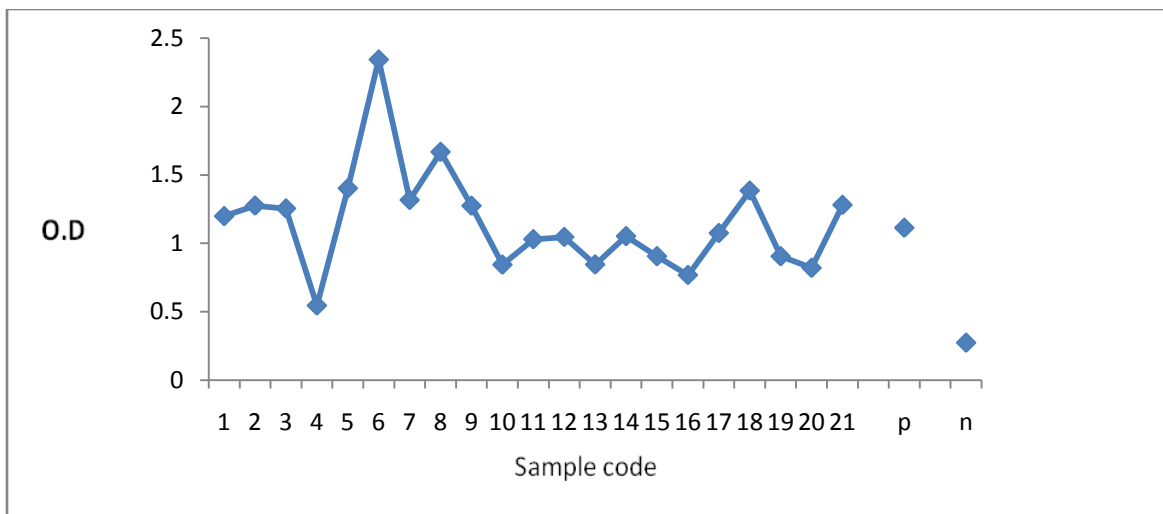
As per the protocol, the titres at 1:150 dilutions were evaluated and compared with the value obtained after multiplying the negative titre at 1:50 by a factor of 2.5 which came about 0.6 and 0.66 in experiment III and experiment IV respectively. Only 55.81 % of the samples tested positive as per the titre 1:150 dilution. The interpretation was carried out on the basis of recommendation of manufacturer (kit protocol).

Further to estimate the titre in International Units (IU), the titres at 1:150 dilutions were converted with the standard (1.83) of FAVN test. At First the K-factor was obtained by dividing the positive titre at 1:50 by 1.83 Standard. which was 0.60 and 0.66 for experiment I and experiment II respectively. Based on the calculated IU values obtained for each serum samples, 79% of the sera samples of the vaccinated dogs belonging to the various age groups showed titre above 0.5 IU/ $\mu$ l. The results of which are described in Table 11.



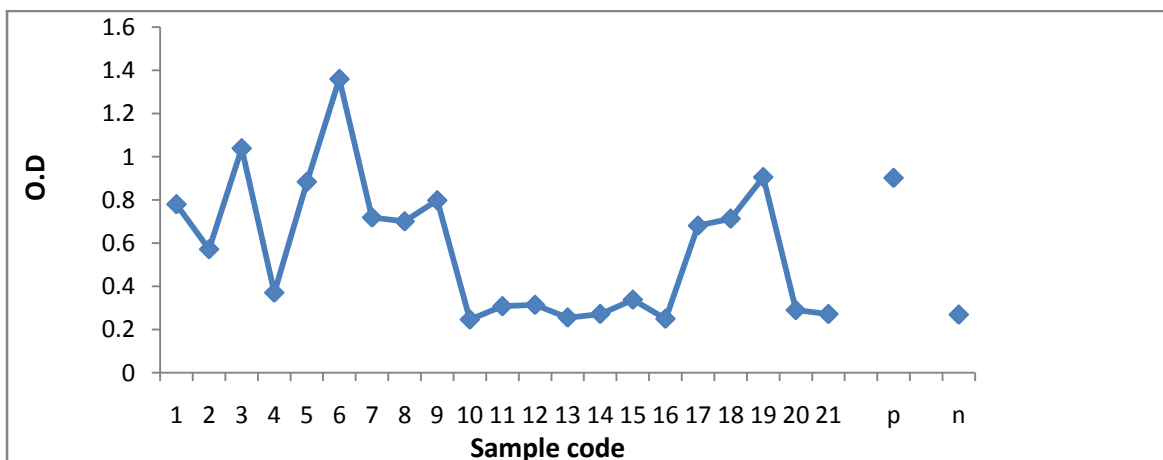
**Fig. 1: The figure depicting O.D values and titres obtained by quantitative I-ELISA in experiment III at dilutions 1:50, 1:150, 1:450 and 1:1350**

The Scatter plot (fig.1) shows the linearly declining regression rate of antibodies present in the sera samples with subsequent dilution rates (1:50, 1:150, 1:450 and 1:1350). The line joining the 4 sets of the titre as depicted in the figure is the Line of Regression.



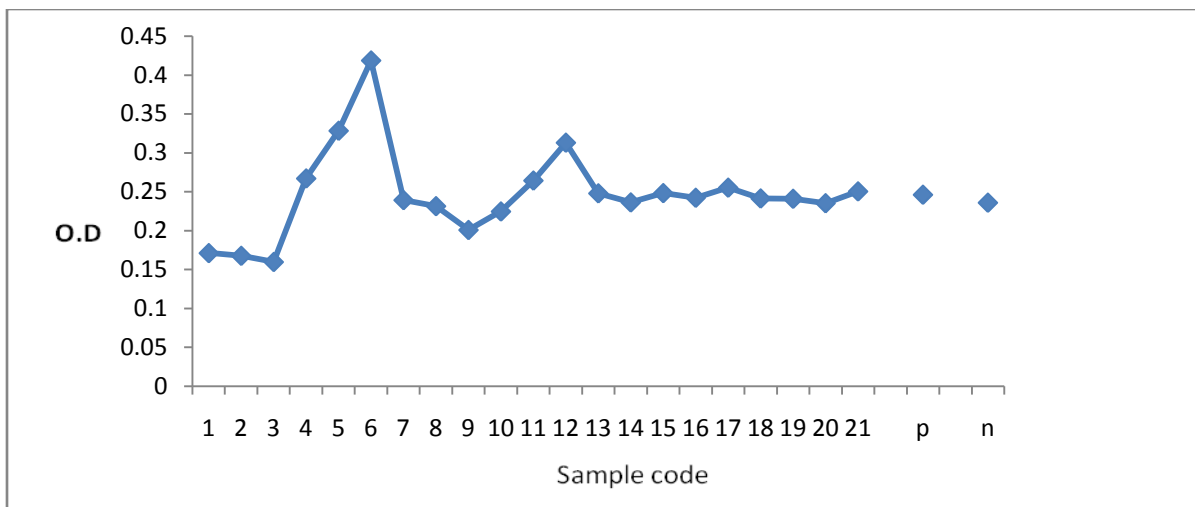
**Fig. 2: The graphical representation of titres of the positive samples at 1:50 dilution in experiment III through Scatter plot**

In the figure above, all of the sera samples revealed positive results by taking the cut off O.D (0.6 cut-off) which was calculated according to the kits protocol.

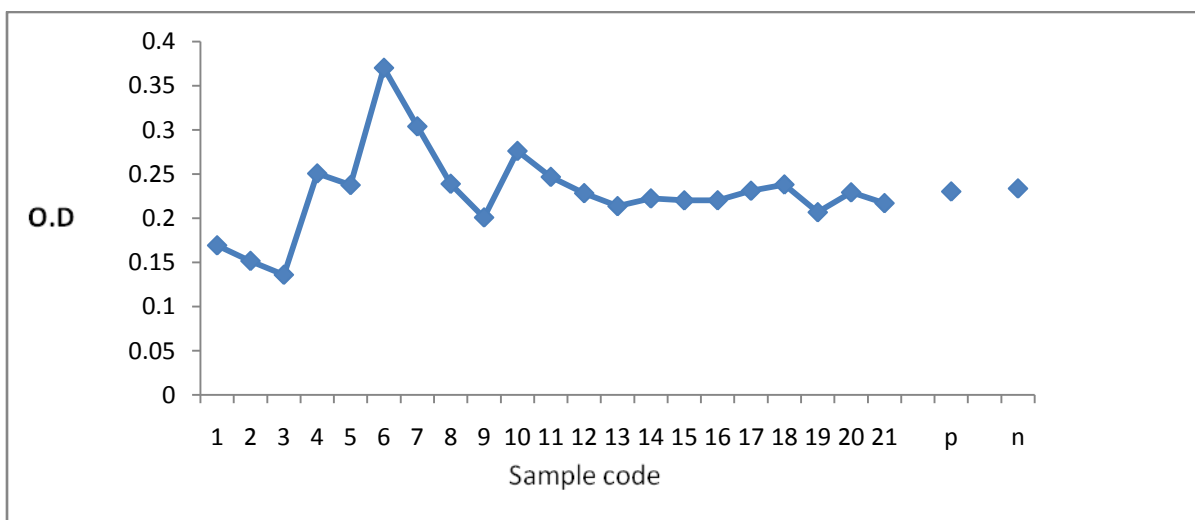


**Fig. 3: The graphical representation of titres of the positive samples at 1:150 dilution in experiment III through Scatter plot**

All the O.D values obtained for samples (1:150) were evaluated by comparing with the calculated cut-off value 0.6.. The O.D of the sera samples above this value were considered to have protective titre. Accordingly 24 samples (55.81%) of samples revealed protective titres to be above 0.6 cut-off value. The same is depicted in Fig. 3.

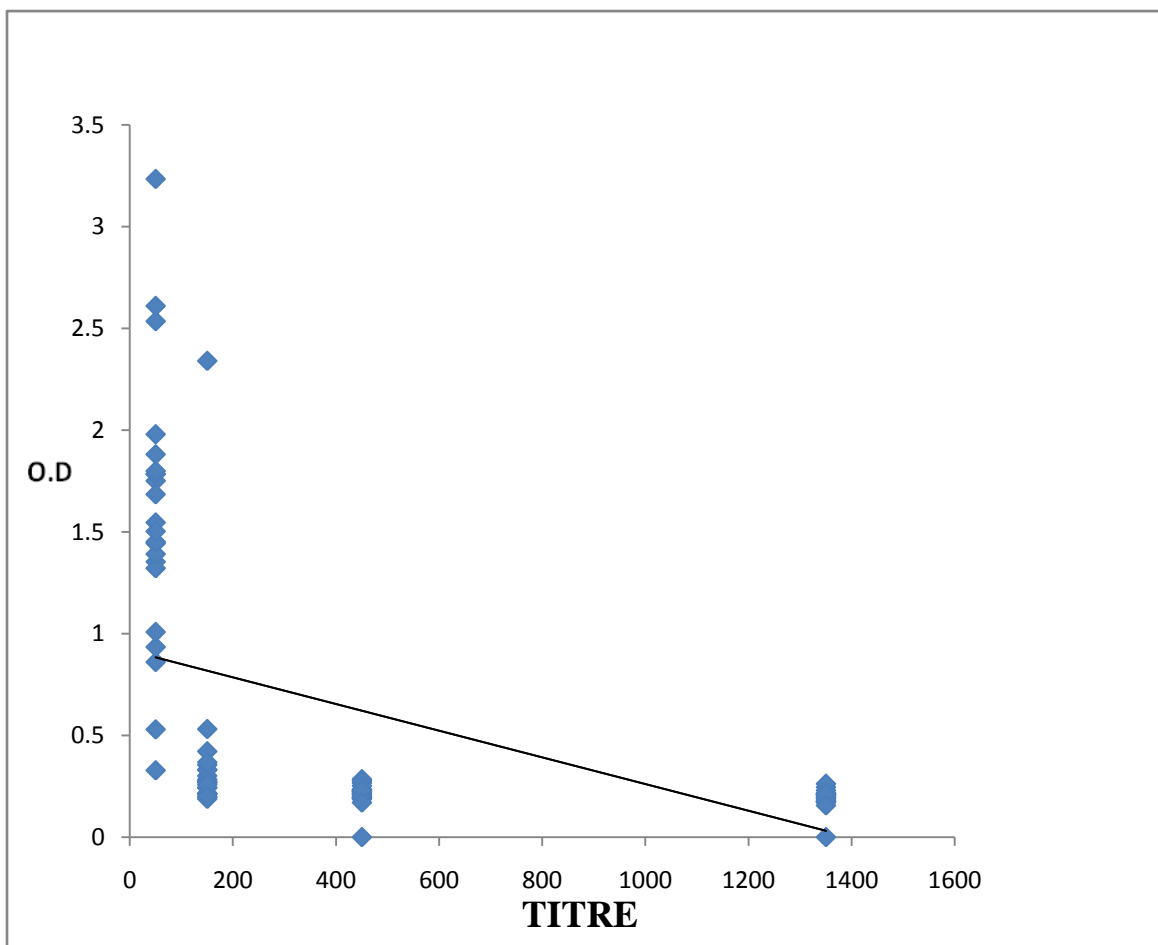


**Fig. 4: The graphical representation of titres of the positive samples at 1:450 dilution in experiment III through Scatter plot**



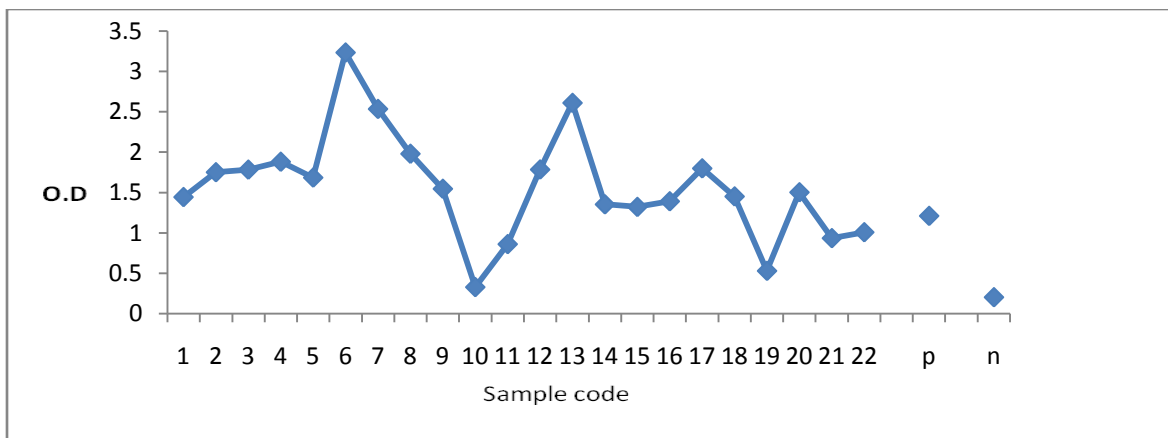
**Fig. 5: The graphical representation of titres of the positive samples at 1:1350 dilution in experiment III through Scatter plot**

Both figures 4 and 5 depict the O.D of the sera samples at dilutions 1:450 and 1:1350, although the evaluation of the positive results as per the recommended protocol was carried out at the 1:150 dilution of the sera samples. The same was shown in fig. 3.



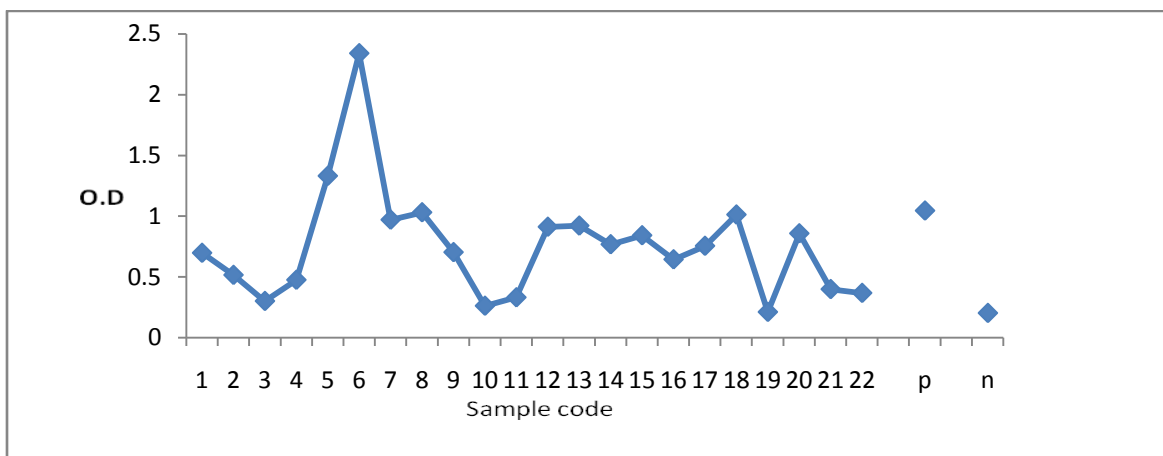
**Fig. 6: The figure depicting O.D values and titres obtained by quantitative I-ELISA in experiment IV at dilutions 1:50, 1:150, 1:450 and 1:1350**

The Scatter plot (fig.6) shows the linearly declining regression rate of antibodies present in the sera samples with subsequent dilution rates (1:50, 1:150, 1:450 and 1:1350). The line joining the 4 sets of the titre as depicted in the figure is the Line of Regression.



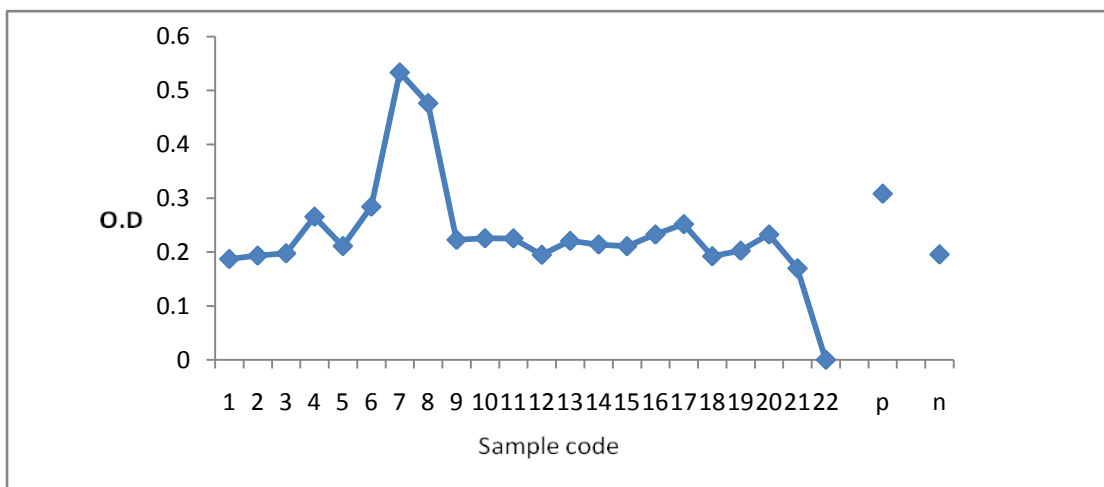
**Fig. 7: The graphical representation of titres of the positive samples at 1:50 dilution in experiment IV through Scatter plot**

In the figure above, all of the sera samples revealed positive results by taking the cut off O.D (0.66 cut-off) which was calculated according to the kits protocol.

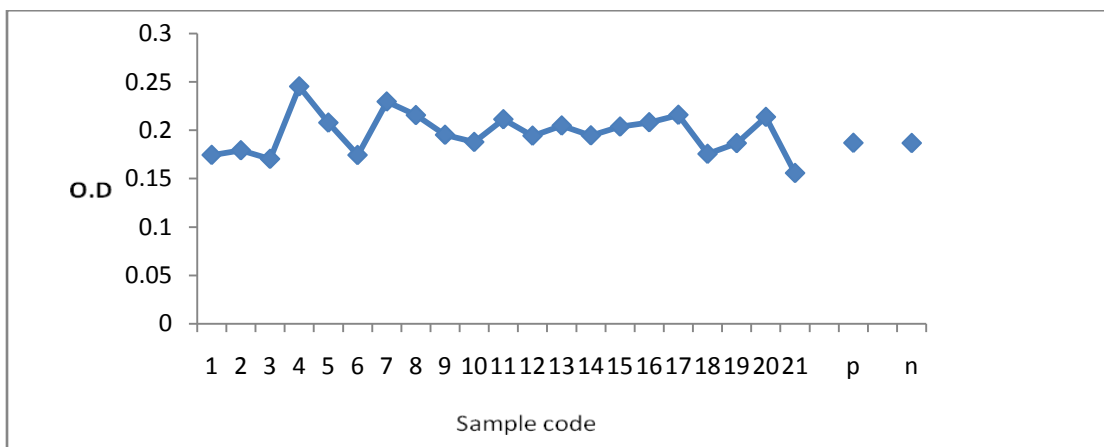


**Fig. 8: The graphical representation of titres of the positive samples at 1:150 dilution in experiment IV through Scatter plot**

All the O.D values obtained for samples (1:150) were evaluated by comparing with the calculated cut-off value 0.6.. The O.D of the sera samples above this value were considered to have protective titre. Accordingly 24 samples (55.81%) of samples revealed protective titres to be above 0.6 cut-off value. The same is depicted in Fig. 8.



**Fig. 9: The graphical representation of titre of the positive samples at 1:450 dilution in experiment IV through Scatter plot**



**Fig. 10: The graphical representation of titre of the positive samples at 1:1350 dilution in experiment IV through Scatter plot**

Both figures 9 and 10 depict the O.D. of the sera samples at dilutions 1:450 and 1:1350, although the evaluation of the positive results as per the recommended protocol was carried out at the 1:150 dilution of the sera samples. The same was shown in fig. 8.

In order to estimate the titres in IU, the titres at 1:150 dilutions were converted with the standard (1.83) of FAVN test as given in the Kits protocol. The K-factor was obtained by dividing the positive titre at 1:50 by 1.83 Standard and the values 0.60 and 0.66 were obtained for experiment III and experiment IV respectively.

In age group upto 3 months, the positive titres in IU ranged between 0.512-0.617 IU/ $\mu$ l and was obtained in 2/5 sera samples in this category. In the age group 4 months ->1year, the positive titres ranged between 0.560-2.267 IU/ $\mu$ l which was recorded in 10/12 sera samples falling in this category. In the age group 1.5 years and above, 20/26 sera samples revealed positive titres range between 0.5018-3.538 IU/ $\mu$ l. Overall 79 percent of the titres of the vaccinated dogs belonging to the various age groups had titres above 0.5 IU/ $\mu$ l. The results of which are presented in Table 11.

**Table 11: Overall Age wise rabies antibody titre level in dogs based on I- ELISA (done quantitatively) cut off level 0.5 IU/ $\mu$ l (based on two experiments)**

Age (Vaccination Schedule category)	No. of animals tested	Titre above 0.5 IU/ $\mu$ l		Titre below 0.5 IU/ $\mu$ l		Protective level (%)
		No.of animals	Titre range in IU/ $\mu$ l	No.of animals	Titre range in IU/ $\mu$ l	
<b>Upto 3 months (Category I)</b>	5	2	0.512-0.617	3	0.4155- 0.452	40
<b>4 months - &gt;1 year (Category II )</b>	12	10	0.56-2.267	2	0.32- 0.4095	83.33
<b>1.5 and above (Category III)</b>	26	22	0.5018- 3.538	4	0.3969- 0.4825	84.61
<b>Total</b>	43	34		9		79.06

\* Figures in the parentheses indicate the percentage value



**PLATE 1: Blood collection in dog**



#### 4.5. ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICE (KAP) OF DOG OWNERS TO CANINE RABIES IN JAMMU

Study population comprised of 200 respondents [130 (65%) urban area dwellers, 70 (35%) rural area dwellers] who were interviewed in the study area. Age of participants ranged between 18 to 65 year group. The interviewed persons were briefed about the purpose of the study and the participation was voluntary. For ethical purposes, the data collected was kept confidential. The details are presented in Tables 12 and 13.

**Table 12: Distribution of the population on the basis of location of residency:**

Location	Number (%) [n=200]
Urban	130 (65)
Rural	70 (35)

n represents total number of respondents

\*Figures in the parentheses represent percentage values

The number of respondents from urban areas were higher (130) compared to rural (70) who were interviewed.

**Table 13: Distribution of the respondents as per age:**

Age Group (years)	Number (%)
18-29	110 (55)
30-41	48 (24)
≥42	42 (21)

n represents total number of respondents

\*Figures in the parentheses represent percentage values

The table depicts higher number of respondents from the 18-29 years of age group (55%) followed by 30-41 years (24%) and ≥42 years old (21%).

#### **4.6 DATA MANAGEMENT AND ANALYSIS**

Data were entered, managed and analysis was carried using SPSS software v.16 and Microsoft Excel 2007.

#### **4.7 DESCRIPTIVE STATISTICS**

Descriptive statistics were calculated for each variable of interest. Bivariate analysis were performed using  $\chi^2$  test to compare the responses to the questions related to the knowledge, attitude and perception of rabies between respondents from urban and rural areas as well as dog owners and non-dog owners. A p-value <0.05 was considered statistically significant.

#### **4.8 STATISTICAL ANALYSIS OF RESPONDENTS DEMOGRAPHIC AND SOCIO-DEMOGRAPHIC CHARACTERISTICS WITH RESPECT TO KNOWLEDGE, ATTITUDE AND PRACTICE**

A total of 200 respondents were interviewed in the survey. Socio-demographic questions related to gender, age of the respondents, educational level, occupation, dog ownership status and total persons in the household were asked. Statistical analysis was carried to evaluate the significance of these socio-demographic characters with respect to knowledge, attitude and practices of the respondents. General linear model was applied to evaluate the results by multivariate analysis of Variance (MANOVA). The results with p-value below 0.05\* was considered to be statistically significant. The median age of the respondents was 40 years (mean 41.5, range 18 to 65).

Table 14 shows the MANOVA analysis of respondents with respect to socio-demographic characteristics.

**Table 14: Multivariate General Linear Model results of the demographic characters of respondents with respect to knowledge, attitude and perception of rabies**

<b>Variable</b>	<b>n (%)</b>	<b>Knowledge (p-value)</b>	<b>Attitude (p-value)</b>	<b>Practice (p-value)</b>
<b>Gender</b>				
Male	152 (76)	<b>0.026*</b>	<b>0.001*</b>	<b>0.000*</b>
Female	48 (24)			
<b>Age</b>				
18-29	110 (55)			
30-41	48 (24)	<b>0.000*</b>	<b>0.000*</b>	<b>0.002*</b>
≥42	42 (21)			
<b>Educational level</b>				
Illiterate	22 (11)			
High school	46 (23)			
Diploma	54 (27)	<b>0.000*</b>	<b>0.015*</b>	0.700
Graduate/above	78 (39)			
<b>Occupation</b>				
Student	18 (9)			
Farmer	24 (12)			
Employee	50 (29)	<b>0.000*</b>	<b>0.002*</b>	0.993
Business	44 (22)			
Dependent/housewife	52 (26)			
<b>Dog ownership status</b>				
Yes	138 (69)	<b>0.000*</b>	<b>0.001*</b>	<b>0.000*</b>
No	62 (31)			
<b>Persons in household</b>				
≤5	110 (55)	<b>0.000*</b>	0.709	<b>0.004*</b>
>5	90 (45)			

n=total number of respondents, \*=implies the significant results with p-value less than 0.05

\* Figures in the parentheses indicate the percentage value

#### 4.8.1 Scoring as per questions regarding Knowledge, Attitude and Practices

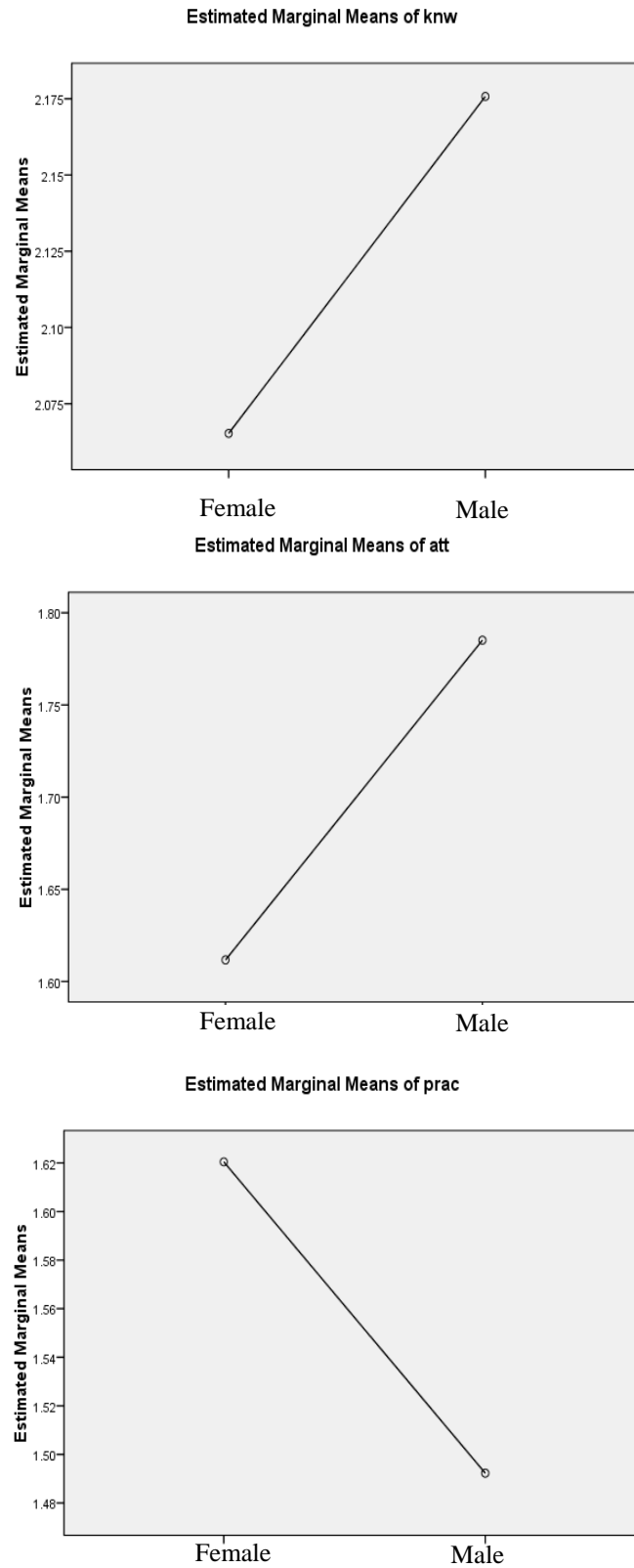
The number of questions to which the respondent positively responded was counted and the score was categorized based on the median. The scores for Knowledge were <4= 42 (21%), 4-6= 124 (62%) and >6= 34 (17%). Similarly for Attitude, scores were 3-7= 168 (84%) and >7= 32 (16%) and for Practices were <4 = 133 (66.5%) and 4-6 = 67 (33.5%).

The data regarding scoring is presented in Table 15. Fig. 11 depicts the MANOVA based graphical representation of association of various demographic characters with respect to KAP.

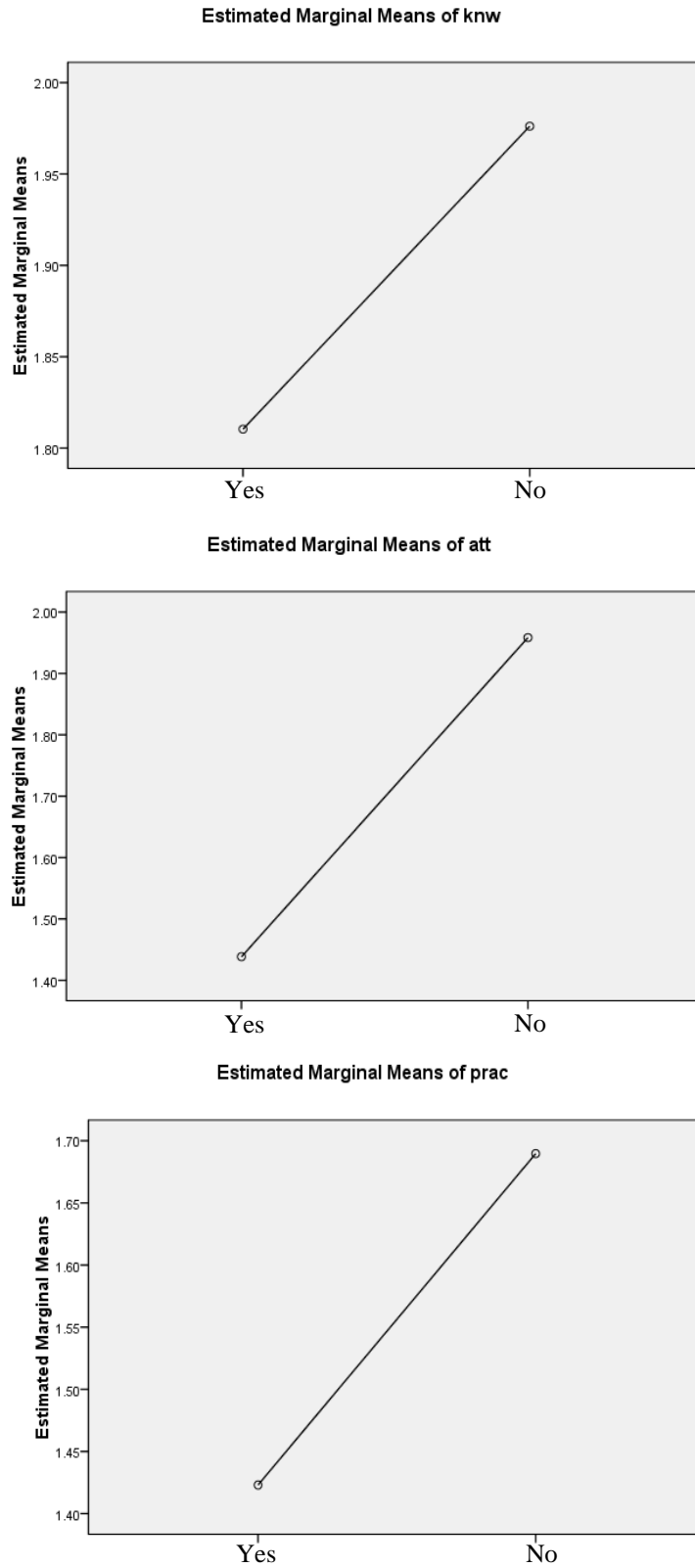
**Table 15: Scoring of the response to questions related to Knowledge, Attitude and Practices about rabies**

SCORING	Number (%)
Knowledge	
<4	42 (21)
4-6	124 (62)
>6	34 (17)
Attitude	
3-7	168 (84%)
>7	32 (16%)
Practice	
<4	133 (66.5)
4-6	67 (33.5)

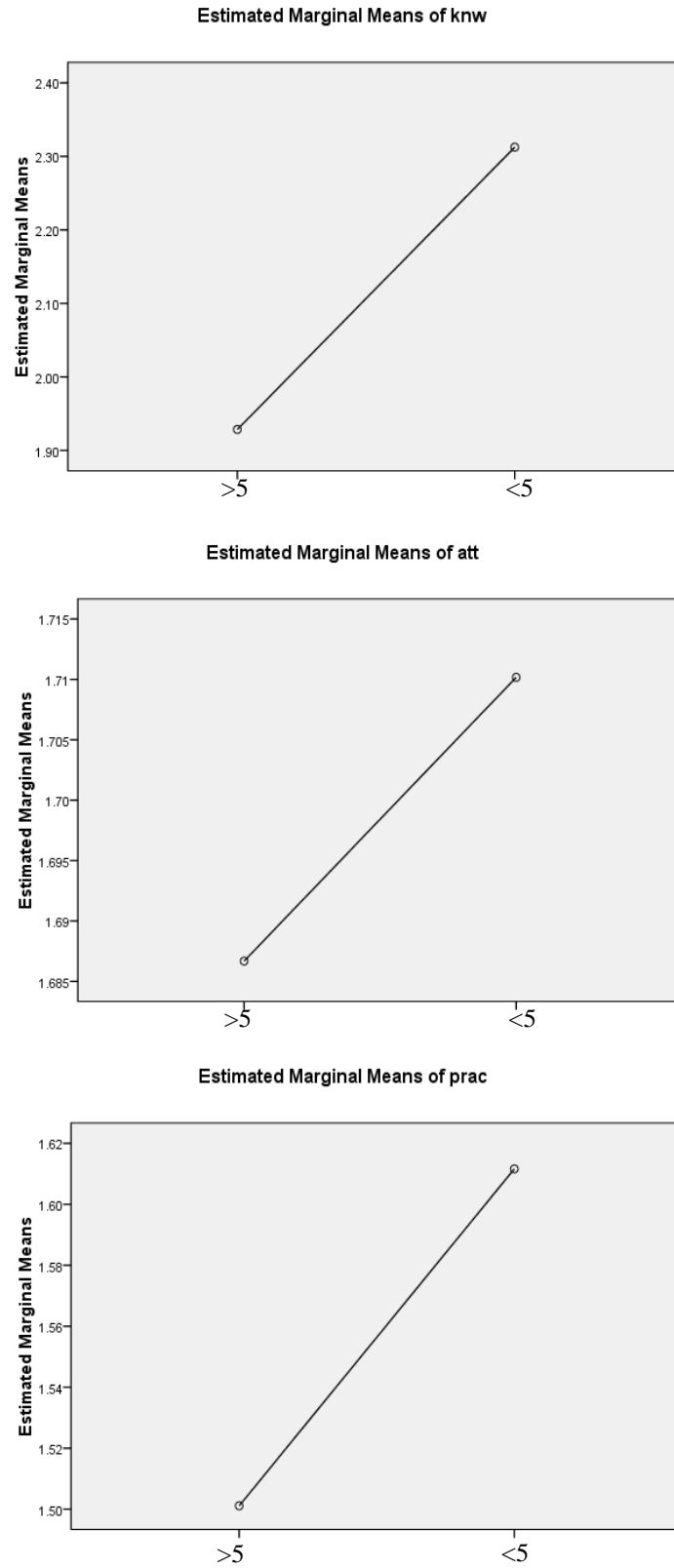
\* Figures in the parentheses indicate the percentage value



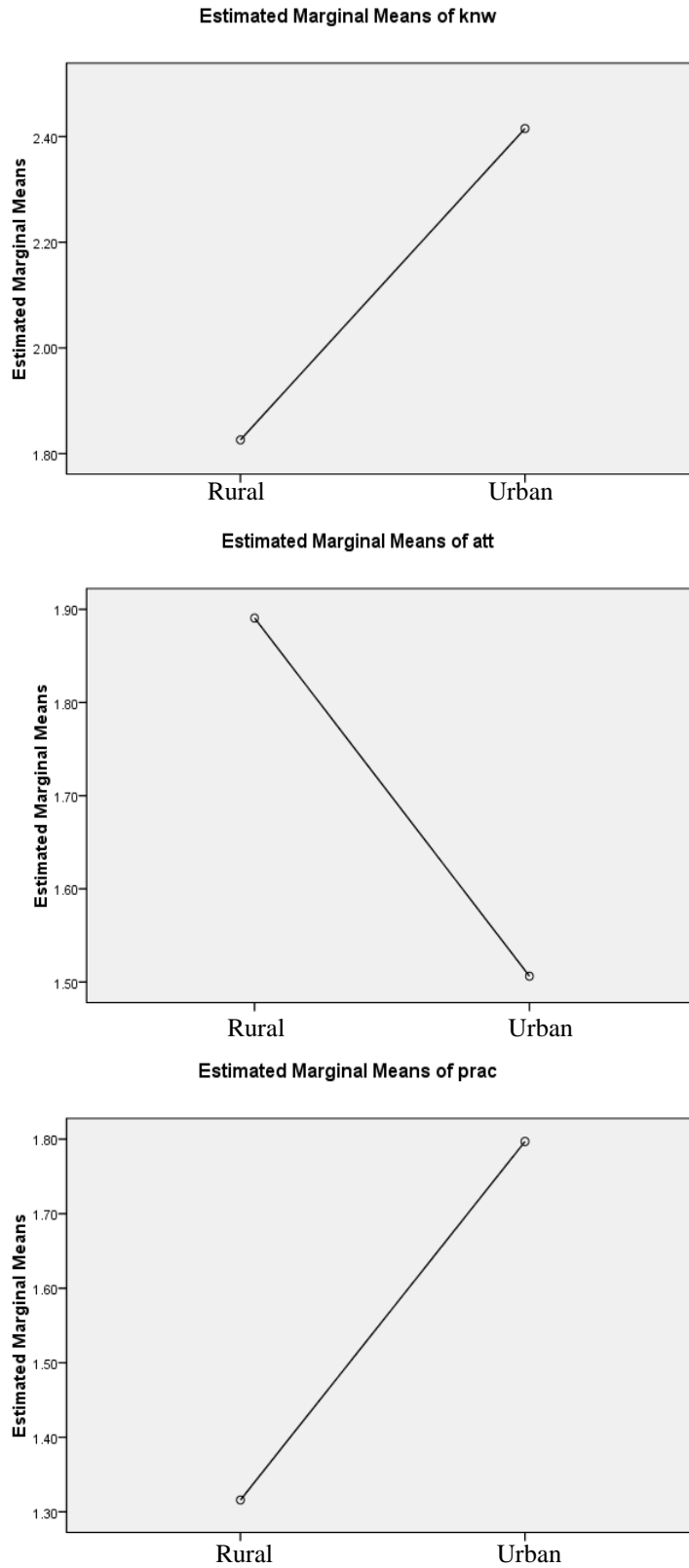
**Fig.11.(a) KAP with respect to Demographic Characters based on MANOVA on the basis of Gender**



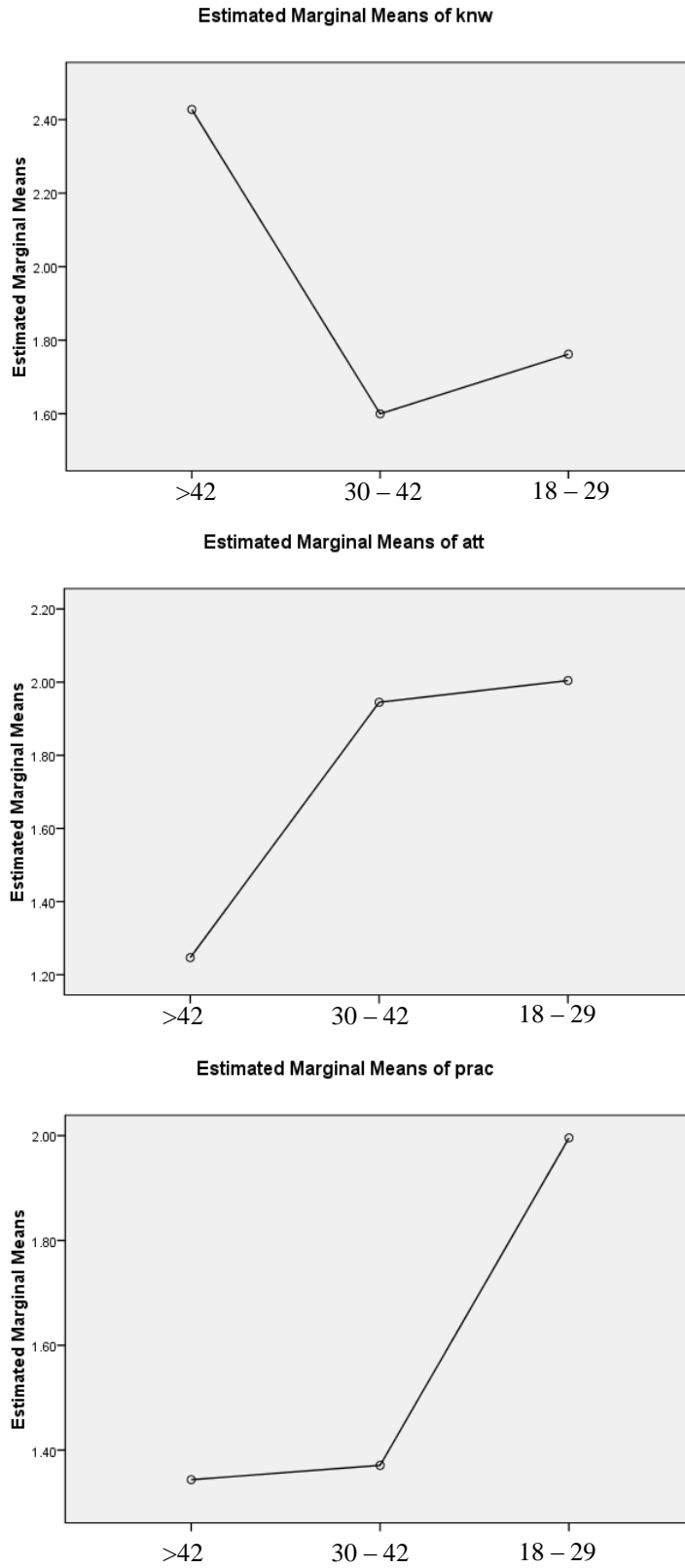
**Fig. 11(b): KAP with respect to Demographic Characters based on MANOVA on the basis of Dog ownership status**



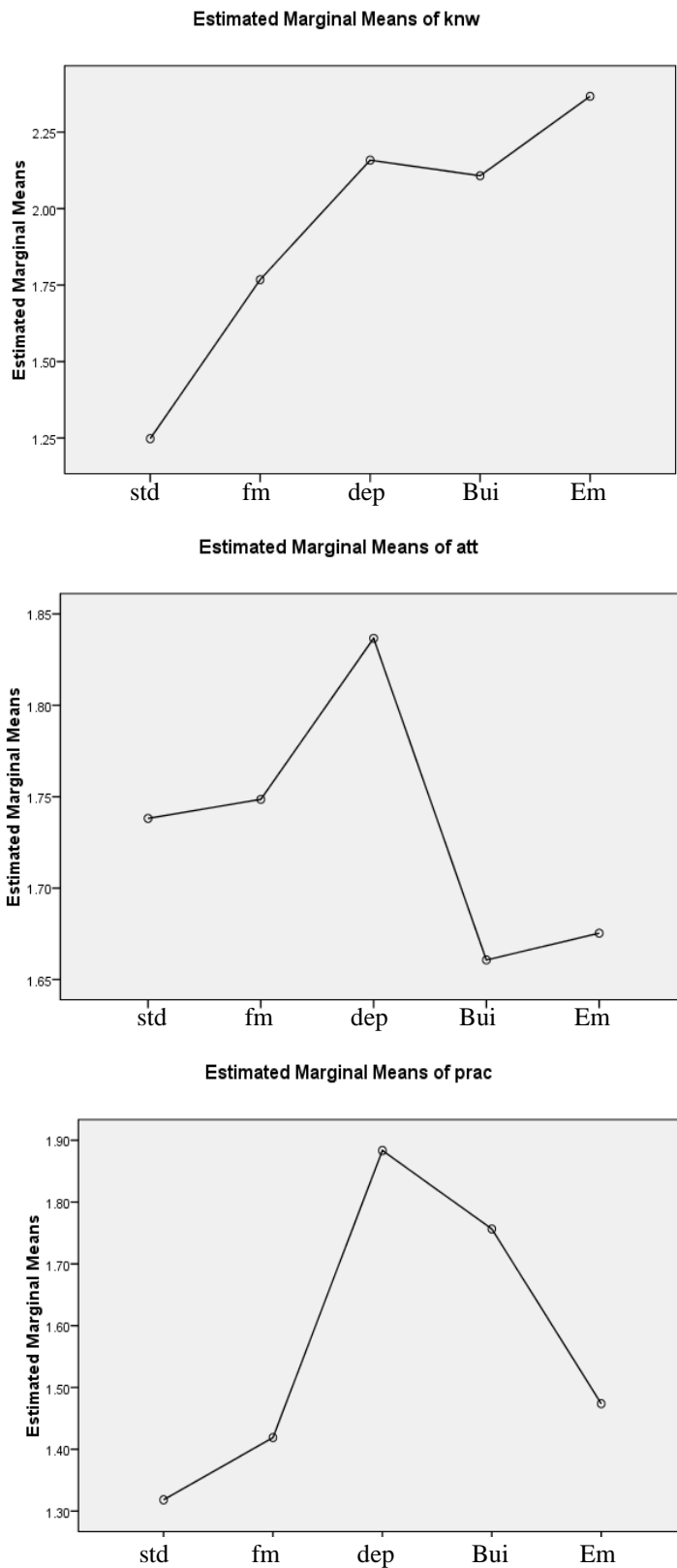
**Fig. 11(c): KAP with respect to Demographic Characters based on MANOVA on the basis of number of Persons in the household**



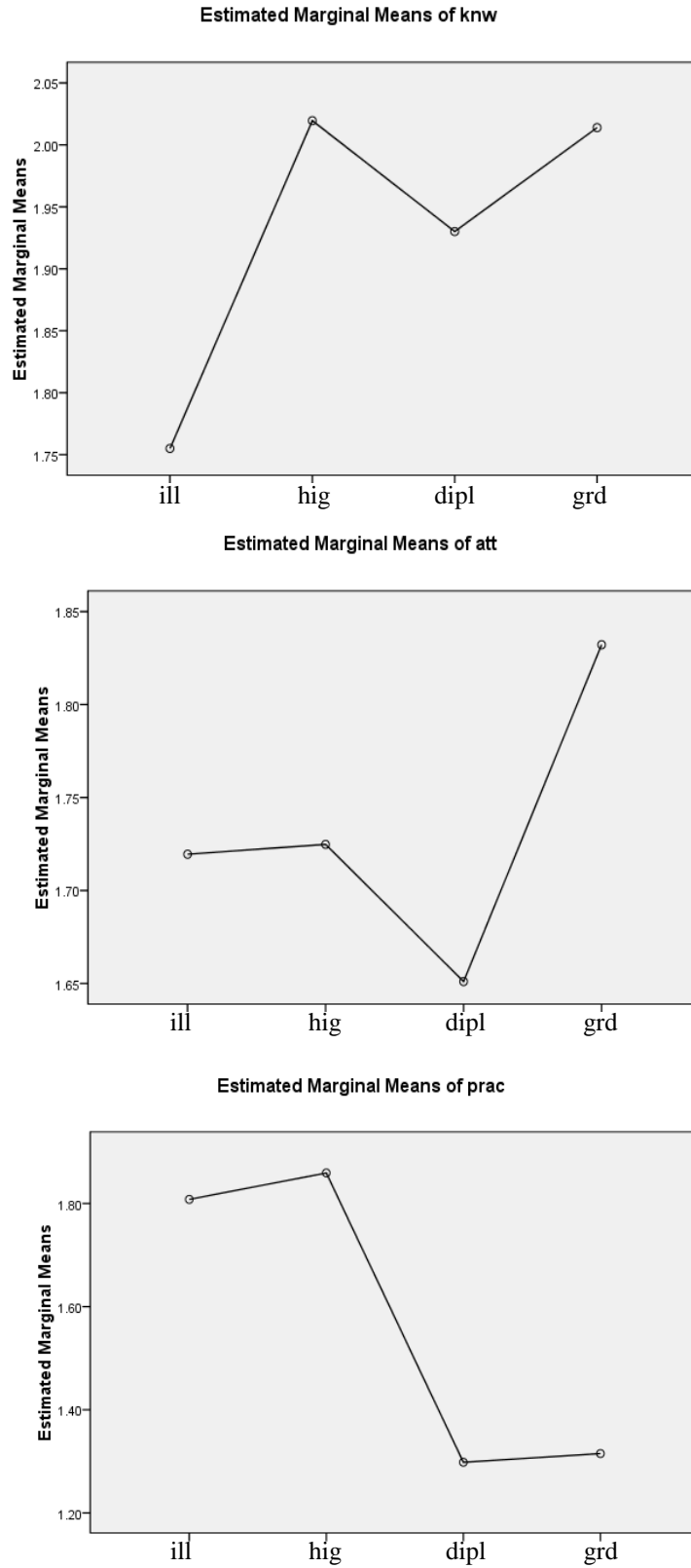
**Fig. 11(d): KAP with respect to Demographic Characters based on MANOVA on the basis of Location (rural/urban)**



**Fig. 11(e): KAP with respect to Demographic Characters based on MANOVA on the basis of Age of respondents**



**Fig. 11(f): KAP with respect to Demographic Characters based on MANOVA on the basis of Occupation of respondents**



**Fig. 11(g): KAP with respect to Demographic Characters based on MANOVA on the basis of Education status**

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**Abbreviations:**

\*std-student, fm-farmer, dep-dependent, Bui-Business, Em-Employed (**Occupation**)

\* ill-Illetrate, hig-High school, dipl-Diploma holders, grd-graduate (**Education**)

#### **4.9 STATISTICAL ANALYSIS OF THE KNOWLEDGE AND PERCEPTION BASED QUESTIONS REGARDING RABIES**

In the Table 16, the results of Bivariate  $\chi^2$  analyses of the respondent's knowledge and perception of rabies and rabies control measures in Jammu are presented. Out of 200 respondents 75.8 percent had heard of rabies and also believed that rabies is a dangerous and fatal disease, transmitted by dog and that can be prevented through dog vaccination. They also believed that there were no locally available methods of treatment for dog bite wound and rabies. Respondents having awareness that animal bite wounds should be washed with soap and water were 64 percent.

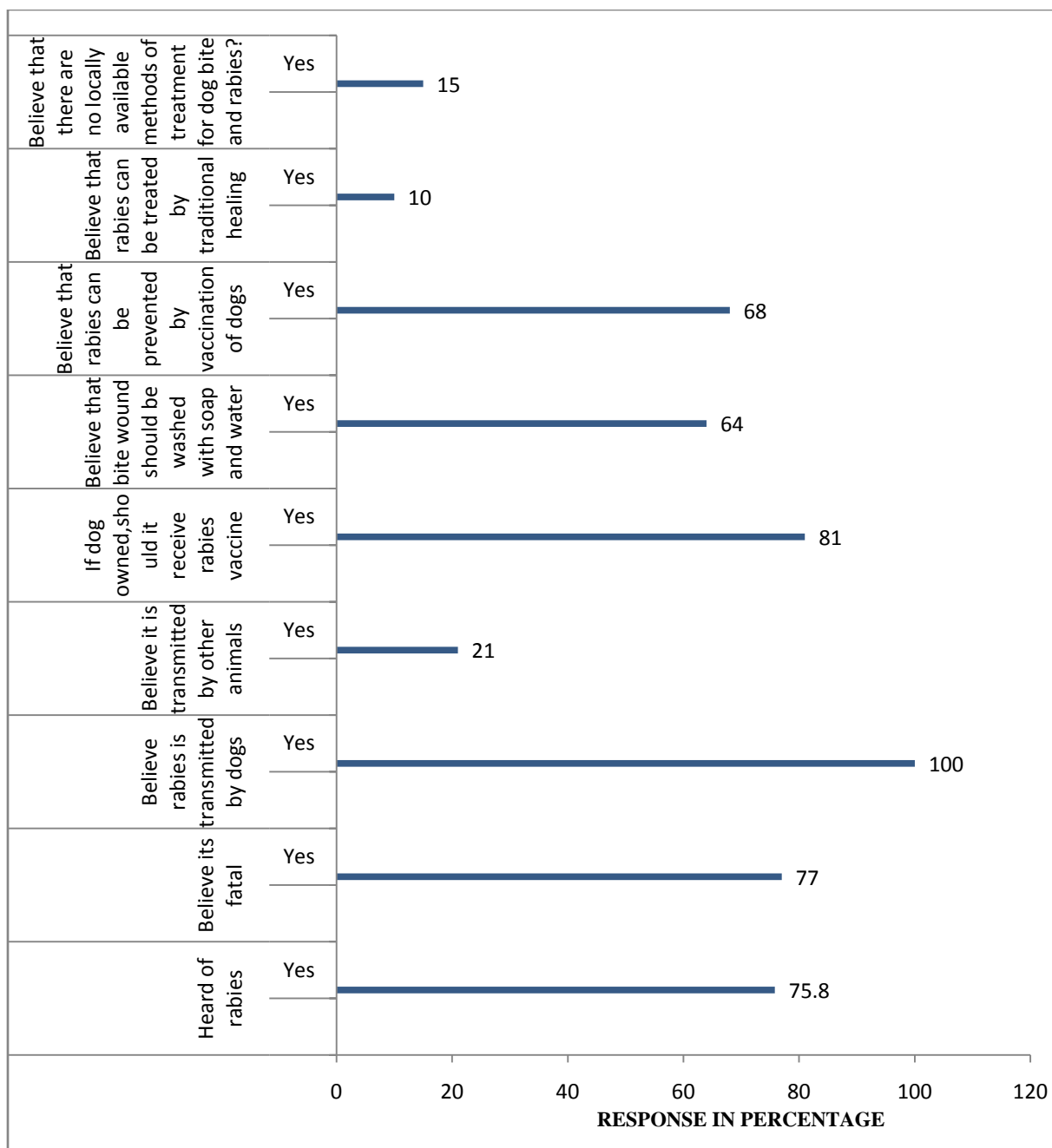
There was significant difference between dog owners and non dog owners with respect to awareness of rabies (p-value=0.004\*), and also believed that it is fatal (p-value=0.000). Similarly, there was significant difference (p-value-0.000\*)between response of the participants from urban and semi-urban areas with respect to the belief that rabies was fatal, believed that rabies can be transmitted by animals other than dogs (p-value=0.009\*). They believed the owned dog should receive rabies vaccine (p-value=0.038\*), and further rabies can be prevented by vaccination (p-value=0.038\*).

In Fig. 12 responses to various questions to dog owners regarding knowledge and perception are presented.

**Table 16: Descriptive and Bivariate  $\chi^2$  analysis of responses to questions related to the knowledge and perception of rabies comparing dog owners with non dog owners as well as respondents living in urban and rural areas of Jammu**

Variable	n%	Dog ownership status (%)		$\chi^2$	O.R(95 %CI)	p-value	Respondent's area of living (%)		$\chi^2$	O.R(95 % CI)	p-value
		Yes	No				Urban	Rural			
Heard of Rabies YES NO	75.8 24.2	95 5	82 18	8.303	4.18	<b>0.004*</b>	91.5 8.5	87.1 12.9	0.862	1.595	0.353
Believe its fatal YES NO	77 23	99 1	80 20	19.207	24.75	<b>0.000*</b>	98.9 1.1	80.9 19.1	18.00	21.43	<b>0.000*</b>
Believe rabies is transmitted by dogs YES NO	100 -	100 -	97 3	3.04	0.00	0.081	97 3	91.6 8.4	2.405	2.963	<b>0.012*</b>
Believe it is transmitted by other animals YES NO	21 79	28 72	19 81	2.253	1.673	0.133	92 8	79 21	6.816	3.055	<b>0.009*</b>
If dog owned, should it receive rabies vaccine YES NO	81 19	82.2 17.8	88 12	1.412	0.629	0.235	94 6	85 15	4.310	2.7625	<b>0.038*</b>
Believe that bite wound should be washed with soap and water YES NO	64 36	84 16	86 14	0.157	0.854	0.692	87 13	82 18	0.954	1.469	0.329
Believe that rabies can be prevented by vaccination of dogs YES NO	68 32	83 17	88 12	1.008	0.659	0.315	94 6	85 15	4.310	2.7625	<b>0.038*</b>
Believe that rabies can be treated by traditional healing YES NO	10 90	10 90	17 83	2.098	0.542	0.147	11 89	14 86	0.411	0.759	0.521
Believe that there are no locally available methods of treatment for dog bite and rabies? YES NO	15 85	13.4 86.6	15 85	0.166	0.874	0.684	13.7 86.3	84.6 15.4	1.008	0.028	0.864

n=total number of respondents, \*=implies the significant results with p-value < 0.05



**Fig. 12: Bardigram showing the response to the questions related to knowledge and perception of rabies vis-a-vis its control and prevention**

#### **4.10 STATISTICAL ANALYSIS OF THE RESPONSES TO ATTITUDE RELATED QUESTIONS AND PERCEPTION ABOUT RABIES**

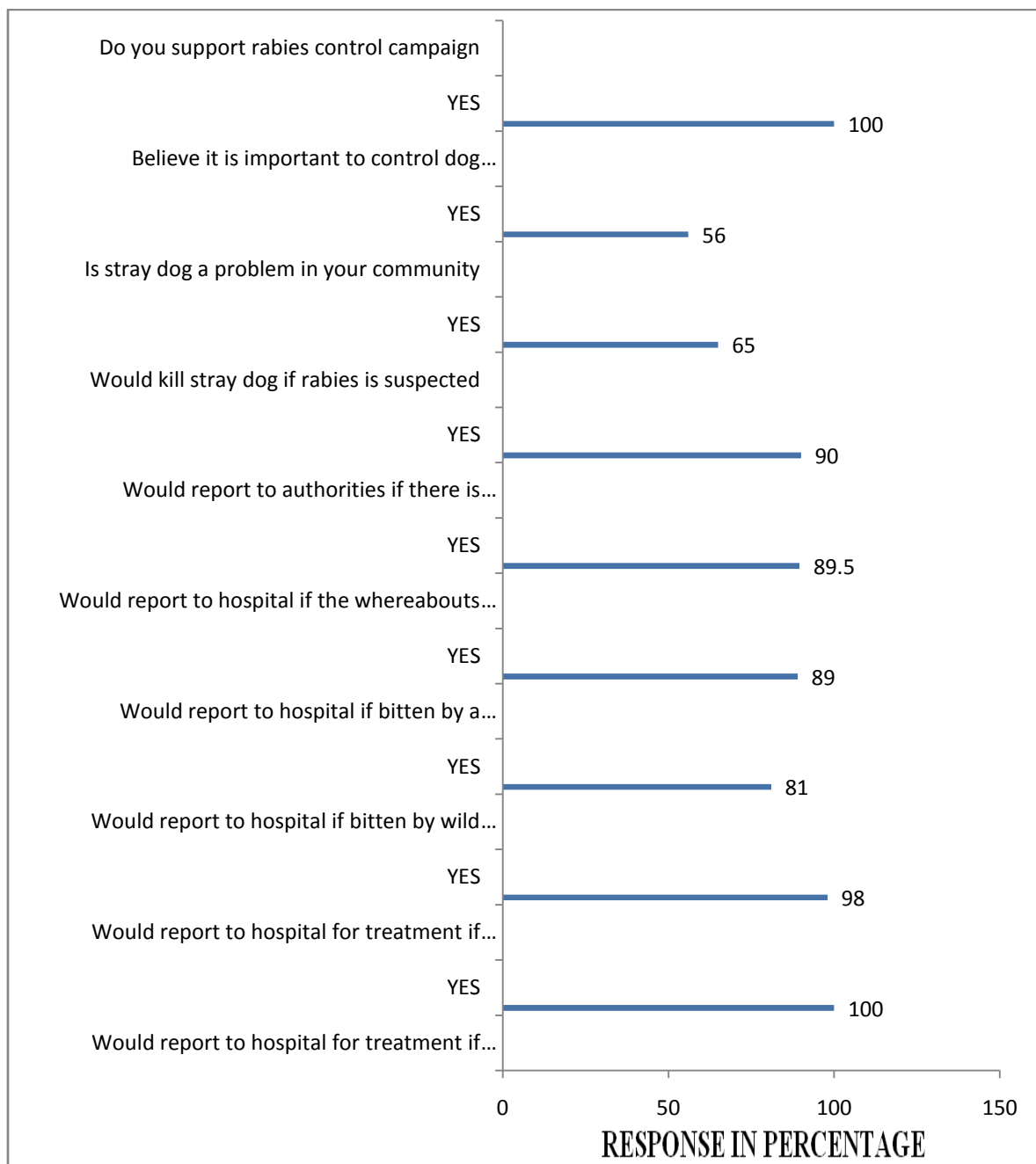
In table 17, the results of bivariate  $\chi^2$  analyses of the respondent's attitude and perceptions of rabies and rabies control programme in Jammu are presented. All the respondents (100%) conveyed that they would report to hospital for treatment if bitten by a stray dog, owned dogs, wild animals and vaccinated dog. While comparing the responses of Urban and Rural dwellers, had significant response (p-value=0.015\*) about reporting to hospital for treatment if bitten by owned dog, bite by wild animals (p-value=0.038\*), about reporting to authorities if there is suspected outbreak of rabies in the community (p-value=0.037\*) and would kill stray dog if rabies was suspected (p-value=0.016\*).

Figure 13 depicts various answers of dog owners to the questions related to knowledge and perception.

**Table 17: Descriptive and Bivariate  $\chi^2$  analyses of responses to questions related to the attitude and perception of rabies comparing dog owner and non-dog owner as well as respondents from urban and rural areas of Jammu**

Variable	n%	Dog ownership status of respondents (%)		$\chi^2$	O.R(95 %CI)	p-value	Respondent's area of living (%)		$\chi^2$	O.R(95 %CI)	p-value
		Yes	No				Urban	Rural			
Would report to hospital for treatment if bitten by a stray dog YES NO	100 -	100 -	100 -	-	-	-	100 -	100 -	-	-	-
Would report to hospital for treatment if bitten by owned dog YES NO	98 2	99 1	97 3	1.020	3.090	0.312	94 6	83.4 16.6	5.944	3.130	<b>0.015*</b>
Would report to hospital if bitten by wild animals YES NO	81 19	82.2 17.8	88 12	1.412	0.627	0.235	94 6	85.2 14.8	4.310	2.723	<b>0.038*</b>
Would report to hospital if bitten by a vaccinated dog YES NO	89 11	92 8	86 14	1.839	1.873	0.175	89 11	79 21	3.72	3.774	0.054
Would report to hospital if the whereabouts of biting animal are not known YES NO	89.5 10.5	91 9	88 12	0.479	1.378	0.489	93.5 6.5	85.6 14.4	2.684	2.729	0.101
Would report to authorities if there is suspected outbreak of rabies in the community YES NO	90 10	93 7	87 13	2.00	1.985	0.157	95.6 4.4	88.4 11.6	4.348	2.921	<b>0.037*</b>
Would kill stray dog if rabies is suspected YES NO	65 35	73 27	62 38	2.758	1.657	0.097	78 22	62.5 37.5	5.858	2.129	<b>0.016*</b>
Is stray dog a problem in your community YES NO	56 44	62.5 37.5	51 49	2.649	1.594	0.104	70.8 29.2	51 49	4.290	2.332	<b>0.038*</b>
Believe it is important to control dog population in Jammu YES NO	100 -	100 -	100 -	-	-	-	100 -	100 -	-	-	-
Do you support rabies control campaign YES NO	100 -	100 -	100 -	-	-	-	100 -	100 -	-	-	-

n=total number of respondents, \*=implies the significant results with p-value less than 0.05



**Fig. 13: Bardigram showing the response towards the questions related to attitude regarding rabies and perception about control and prevention of rabies**

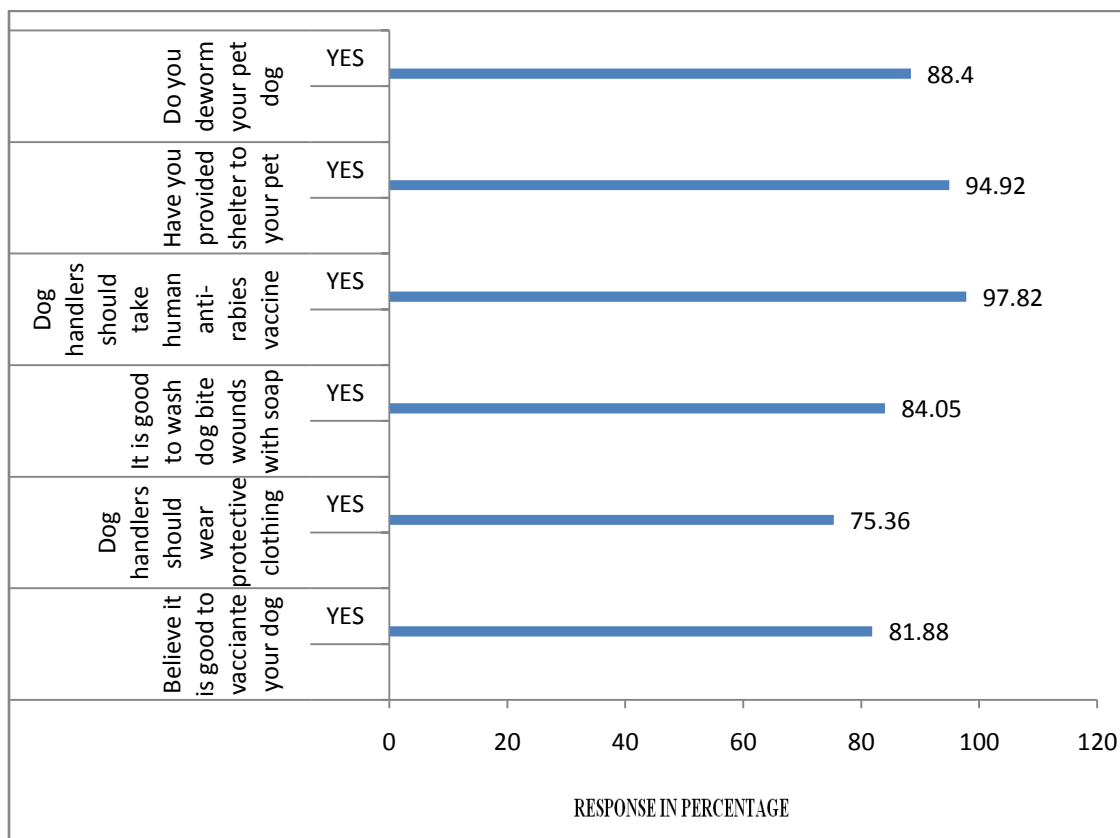
#### 4.11 ANALYSIS OF THE PRACTICE QUESTIONS PERTAINING TO RABIES

The practices were followed by only those respondents who had dogs as pets and not by those who had no pets. In the table 18 and Fig. 14 are presented the frequency of dog owners' response to questions regarding the rabies related practices (prevention and control) in Jammu.

**Table 18: Frequency of response of dog owners to various questions regarding practices**

Questions	Frequency (%)
Believe it is good to vaccinate your dog	
Yes	113 (82.2)
No	25 (17.8)
Dog handlers should wear protective clothing	
Yes	104 (75.53)
No	34 (24.47)
It is good to wash dog bite wounds with soap	
Yes	116 (84)
No	22 (16)
Dog handlers should take human anti-rabies vaccine	
Yes	135 (98)
No	3 (2)
Have you provided shelter to your pet	
Yes	131 (95)
No	7 (5)
Do you deworm your pet dog	
Yes	122 (89)
No	16 (11)

\* Figures in the parentheses indicate the percentage value



**Fig 14. Bardigram showing the response towards the questions related to practice and perception of rabies regarding control and prevention**



**Plate 4: Face to face interview of the people for KAP**

Chapter-V

# Discussion

Rabies is acutely fatal encephalitis caused by a neurotropic virus belonging to the family Rhabdoviridae and genus *Lyssavirus*. The virus is almost always transmitted to human through infected mammalian saliva. Rabies is inoculated to a wound by an infected animal bite. Since infection is established in the CNS, the outcome is almost always fatal (Rad *et al.*, 2009). Rabies prevention in both humans and animals is possible by vaccination. The domestic dog (*Canis familiaris*) is the major reservoir in rabies-endemic Asia and Africa (Harry *et al.*, 1984; WHO, 2005). The vaccination of dogs remains most cost-effective option for rabies control in these regions *where* and herd immunity is achievable when up to 70 percent of dogs are immunized against the disease (Dzikwi *et al.*, 2011). Vaccination against rabies virus is a highly effective method of preventing the disease in humans and animals (Jakel *et al.*, 2008).

Several studies have recognized that the dog rabies control relies on mass vaccination of dogs which inhibits rabies transmission cycle (Perry and Wandeler, 1993). Mass vaccination has been practiced successfully in Western Europe and North America (Wandeler, 2000; WHO, 2004), showing that the disease can be controlled and eliminated by vaccination of reservoir animal population. The voluntary routine dog rabies vaccinations at private, University or government Veterinary Clinics places responsibility on individual dog owners to bring their dogs to the clinic, or to book for ambulatory services. Detection and quantification of antibodies is used primarily for the detection of the immune status of humoral immunity after anti-rabies vaccination (Ondrejko *et al.*, 2015).

Several serological tests for the detection of rabies virus neutralization antibodies have been described. Indirect ELISA tests (Enzyme-Linked Immunosorbent Assay) which incorporate rabies glycoprotein/anti-human immunoglobulin/enzyme conjugates have been in use for both human and animal post-vaccination rabies antibodies titration (Piza *et al.*, 1999). The quantitation of anti-rabies antibodies is considered to be of great

importance and is frequently employed to determine the immune status in case of animals and human beings at pre-exposure and post-exposure treatment. World Health Organization (WHO 1992) recommended standard of 0.5 IU/ $\mu$ l as a protective titre. The detection of antibodies is also useful in rabies diagnosis late in the course of disease (Crepin *et al.*, 1998). ELISA based on the detection and titration of the anti-glycoprotein antibodies may also be used in rabies diagnosis, being simple, safe, rapid and sensitive method for detecting rabies antibodies (Cliquet *et al.*, 2003).

WHO guidelines on rabies post-exposure prophylaxis (PEP) recommend three important aspects of the treatment immediately following exposure to rabid animals: thorough washing of the bite wound with water and soap or detergent, or water alone; administration of rabies vaccine; and infiltration of rabies immunoglobulin into and around the wound (WHO, 1992; 2010). However, in reality people in developing countries, particularly the poor sections of the society, may not receive these life-saving treatments either because the PEP treatment is expensive and not readily available or because people may not visit the hospital for treatment owing to lack of knowledge about rabies (Kayali *et al.*, 2003; Knobel *et al.*, 2005; Hampson *et al.*, 2008).

An understanding of community's knowledge, attitude and perception of rabies assumes importance because it influences the post-exposure treatment seeking behavior (Matibag *et al.*, 2008). Moreover the community support is essential for rabies prevention and control programmes (Kayali *et al.*, 2003). Several studies have been conducted to understand knowledge, attitude and practices for rabies in some parts of India (Agarvval and Reddaiah, 2003; Singh and Choudhary, 2005; Ichhpujani *et al.*, 2006), Sri Lanka (Matibag *et al.*, 2007; Matibag *et al.*, 2008; Matibag *et al.*, 2009), and in North America (McGrill *et al.*, 1997; Goodwin *et al.*, 2002). These studies demonstrated a variably high level of people's awareness regarding rabies. A few other studies about knowledge and perception of rabies risk amongst travelers in rabies-endemic countries are on record (Altmann *et al.*, 2009; Piyaphanee *et al.*, 2010). Such studies have found dangerous vague practices such as applying chilli and turmeric powder, lime, kerosene oil, herbal paste or salt on the dog bite wound, or perform folk remedies at home rather than seeking conventional treatment from health facilities

(Agarvval and Reddaiah, 2003; Singh and Choudhary, 2005; Ichhpujani *et al.*, 2006; Sudarshan *et al.*, 2006; Matibag *et al.*, 2008).

The presented study was conducted to assay the levels of protection achieved in dogs of various age groups after primary and secondary booster vaccination against rabies and to assess the risk of exposure to rabies by analyzing the rabies related knowledge, attitudes and practices of dog owners as well as non-dog owners and also on the basis of location (urban/rural).

In the present study, upon analysis of 180 serum samples of vaccinated dogs, an overall prevalence (qualitative) of 49.44percent was observed (Table 4). The quantitative analysis, out of the positive samples (43), rabies virus specific antibodies (0.5 IU/ $\mu$ l) were detected in 34 (79.06%) serum samples. Whereas 20.94 percent vaccinated dogs did not reveal protective anti-rabies titre (Table11).The results of our findings are comparable to Mugale *et al.*, 2012 who found rabies virus specific antibodies (0.5IU/ $\mu$ l) in 70.55 percent (115/163) cases. Whereas 29.44 percent vaccinated dogs did not reveal protective rabies titre in their study.

In our study, there was lack of proper protective antibody levels in the vaccinated dogs done through quantitative I-ELISA (Category I-40%, Category II-83.33%, and Category III-84.61%) against rabies which were similar to the reports by Tepsumathanon *et al.* (1999) in Bangkok (4/9 or 44.44% HIV patients showed neutralizing antibodies titres above the minimal WHO-recommended level of 0.5 IU/ $\mu$ l and Sage *et al.* (1993) in Alaska (27% of dogs bled at 3 months, 24% at 6 months and in 33% one year after the primary vaccination). In contrast, Chomel *et al.* (1988) had reported 97 percent of the animals with titers above 0.5 IU/ $\mu$ l after vaccination. The lower levels of antibody titre in our study may be attributable to nutritional status, parasitic infestations etc as recorded to the poor immune response in accordance with the reports presented by Sage *et al.* (1993). However, in the present study protective antibody titer on an average was 79.06 percent (34/43) in vaccinated dogs. In contrast, Feyssaguet *et al.* (2006) and Servat *et al.* (2006) found the sensitivity of ELISA 98.6% and specificity 99.4%. Whereas, Servat *et al.* (2006) found a kit highly specific (> 98%) using a cut off level 0.5 IU/ $\mu$ l.

On the basis of age, in case of Qualitative I-ELISA; overall prevalence of anti-rabies antibodies was observed as Category I (38.09%), Category II (39.65%) and Category III (56.43%) (Table7) where as in case of Quantitative I-ELISA; overall prevalence of anti-rabies antibodies was observed as Category I (40%), Category II (83.33%) and Category III (84.61%) (Table11).

A higher sero-prevalence was observed in males 66 (50.38%) as compared to females 23 (46.93%) (Table 8). The lower antibody titre in females may be due to breeding season stress of bitches followed by immune suppression.

The results of cross-sectional study conducted to understand community knowledge, attitudes and perception of rabies and investigating such factors influencing owners knowledge and perceptions about rabies. The present study seems to be the first such study conducted to understand the KAP of rabies in Jammu about canine rabies.

It was taken care that the respondents were interviewed both from urban and rural areas. Age group of people ( $\geq 42$  years), sex (males), education level (graduate), occupation (employee and businessman), persons in household ( $<5$ ), dog ownership status (yes) and location (urban) were found to be significant (p-value  $<0.05$ ) with respect to questions related to knowledge, attitude and practice. The study showed results which were similar to that conducted by Tenzin *et al.*, 2012 in Bhutan who had reported the majority of the respondents to possess high level of knowledge, and attitude and perception of rabies, and has a positive attitude towards the prevention and control. On the basis of regression model observed that better knowledge about rabies with respect to gender, educational level and dog ownership status of the respondents.

Present KAP studies revealed 75.8 percent having heard of rabies and 64 percent were aware to wash bite wound with soap and water. Understanding community knowledge through this study revealed high level of awareness among the respondents with dog ownership which may be due to endemicity of rabies, awareness through the news media about rabies. Findings from this study are in consonance with those from other studies in the south Asia that demonstrated a high level of knowledge of rabies and its transmission (Agarvval and Reddaiah, 2003; Sharma, 2005; Ichhpujani *et al.*, 2006;

Matibag *et al.*, 2008; Matibag *et al.*, 2009). However, the current study also identified some knowledge gaps: as some of the respondents (24.2%) had not heard of rabies and its mode of transmission especially the rural area dwellers indicating lack of rabies awareness education in such parts of Jammu.

Understanding the community attitude and perceptions of treatment-seeking behaviours is considered important for rabies prevention in humans (Matibag *et al.*, 2008). Immediate post exposure prophylaxis (PEP) is required to neutralize the rabies virus in the wound before it spreads into the central nervous system and brain (Warrell and Warrell, 2004; WHO, 2010). This study showed good treatment-seeking behaviours as a majority of the respondents would report to the hospital for animal bite wound treatment. However, such reporting about the animal bite wounds to the hospital were higher for in owners of dogs, and those from urban area.

These findings are comparable with studies reporting that a large number of people visited the hospitals for rabies PEP following dogs bites, touching/feeding of rabid animals and ingestion of meat and dairy products derived from rabid animals in Bhutan (Tenzin *et al.*, 2011). This evidence supports the current finding that the people in urban area have good health-seeking behaviours. It is possible that children would often interact with dogs resulting in dog bite injuries, but probably do not report the incident to their parents or to the hospital owing to lack of awareness of rabies (Dodet *et al.*, 2010). Furthermore, studies in other countries have shown that children are more often bitten on the head and neck, which carries a much higher risk than bites to other parts of the body (Pancharoen *et al.*, 2001; Cleaveland *et al.*, 2002; Knobel *et al.*, 2005). Further studies should be conducted to confirm this proposition, and if found to be correct, awareness and education should be planned targeting children.

Bivariate  $\chi^2$  analysis was conducted with respect to dog ownership status and location of respondents. The results of study were comparable with those of Tenzin *et al.*, 2012. A significant difference was found between dog owners and non-dog owners with respect to awareness of rabies (p-value=0.004\*), and belief that it is fatal (p-value-0.000\*). Significant difference (p-value-0.000\*) between response of the participants from urban and semi-urban areas with respect to the belief that rabies is fatal. Significant

results that rabies is transmitted by other animals other than dogs (p-value=0.009\*), if dog owned it should receive rabies vaccine (p-value=0.038\*) and believe that rabies can be prevented by vaccination of dog (p-value=0.038\*).

The study results shows that male respondents had better knowledge about rabies but they are less likely to report animal bite cases to the hospital. This is not surprising because it is well documented that compared to women, men in general have limited contacts with physicians and seek less healthcare services (Mansfield *et al.*, 2003; Galdas *et al.*, 2005; Smith *et al.*, 2006). It is assumed that several factors might be involved in men's decisions, including masculine ideologies regarding seeking help when faced with illness or problems (Galdas *et al.*, 2005; Smith *et al.*, 2006). This is comparable to field observations that of the 12 human rabies deaths in Bhutan (from January 2006-April 2011), 11 (92%) were males but most (75%) were children under 15 years of age.

The current results also indicate that the attitudes of the respondents were positive: the majority believed in reporting suspected rabies outbreaks in the community to the appropriate authorities for investigation. The majority of respondents also believed that stray dogs were a public health problem in the community and would support a dog population control programme. Community support of, and participation in a rabies control programme is important in order to achieve good coverage of vaccination (>70 %). This is necessary to break the chain of infection and to prevent the maintenance of rabies in the dog population (WHO, 1992; Coleman and Dye, 1996).

From the present study, it may be concluded that secondary booster vaccination of dogs had higher amount of anti-rabies antibodies in serum samples (Category III). There was much more awareness amongst the people who owned dogs and urban area dwellers about rabies and its control and further about measures to be taken in order to avoid health hazard caused due to it.

Chapter-VI

Summary  
and  
Conclusions

## CHAPTER-VI

### SUMMARY AND CONCLUSIONS

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Rabies is a neurological disease of mammals, almost invariably fatal once clinical signs develop. Causes acute, viral encephalomyelitis and affects all warm blooded animals. Being highly fatal, it has a case fatality rate of almost 100 percent. It is caused by neurotropic virus of genus Lyssavirus, family - Rhabdoviridae, and transmissible to all mammals. Over 99 percent of human exposures to rabies result from the bite of domestic dog, *Canis familiaris*

Most of the dogs are not vaccinated against rabies. Although WHO has recommended that 70 percent of dogs must be vaccinated to affect control of rabies in a community. It is not enough just to vaccinate individual dogs, rather - efficacy of the vaccines evidenced by sero-conversion needs to be monitored.

The commensed study was a cross sectional one which was carried out during the period from June, 2016 to April, 2017, with an aim to generate data on the immune response in a cross-section of dogs population reporting of vaccination to various clinical settings. A total of 180 sera samples were taken from dogs which were classified into three main categories viz., Category I, Category II and Category III.

Besides testing the serological response to anti-rabies vaccines, an Epidemiological survey was carried to detect the awareness of the dog owners and non-dog owners with respect to rabies and its control and prevention. The study comprised of the respondents from the Urban or Rural areas.

The present study involved qualitatively and quantitatively detection of the anti-rabies antibodies by I-ELISA in dog serum after vaccination to evaluate its protective effect. Qualitatively only 49.44 percent sero-prevalence of anti-rabies antibodies (n=180) was recorded. Age-wise, upto 3 months - 38.09 percent, 4 months to >1 year - 39.65 percent and 1.5 years and above - 56.43 percent samples revealed anti-rabies antibodies in dog serum. Quantitatively 35/45 sera samples (79.06%) had titres above 0.5

IU/ $\mu$ l. Age-wise, upto 3 months – 40 percent, 4 months - >1 year – 83.33 percent and 1.5 years and above – 84.61 percent samples had protective titres above 0.5 IU/ $\mu$ l . Antibodies against rabies in Females were less (46.93%) compared to males (50.38%).

KAP studies based on 200 respondents revealed that 75.8 percent had heard about rabies and 64 percent were aware of washing wound bite with soap and water. Demographic characters with respect to questions revealed strong association ( $p < 0.05$ ) of educated respondents, having dog ownership status, dwellers of urban area and males with respect to Knowledge, Attitude and Practice.

There was significant difference ( $p < 0.05$ ) between dog owners and non-dog owners with respect to awareness of rabies and believed the rabies to be fatal.

A significant difference between dog owners and non-dog owners with respect to awareness of rabies ( $p$ -value=0.004\*), and believed it to be fatal ( $p$ -value-0.000\*). Significant difference ( $p$ -value-0.000\*) was observed between responses of the participants from urban and semi-urban areas with respect to the questions related to fatal outcome of rabies. Results pertaining to questions that rabies is transmitted by animals other than dogs was found significant ( $p$ -value=0.009\*). Significant information was recorded that owned dog must receive anti-rabies vaccine and also knew that rabies can be prevented by vaccination of dog ( $p$ -value=0.038\*).

All the respondents irrespective of ownership of dogs and location of their residence (urban/rural) believed in reporting to hospital for treatment if bitten by a stray dog, owned dog, wild animals and even through vaccinated dogs in Jammu. Significant value regarding reporting to hospital for treatment if bitten by owned dog ( $p$ -value=0.015\*) and by wild animals ( $p$ -value=0.038\*) was recorded. A significant respondents believed in reporting to authorities if there was suspected outbreak of rabies in the community ( $p$ -value=0.037\*) and would kill rabies suspected stray dog ( $p$ -value=0.016\*).

Overall a comparatively good approach towards Rabies knowledge and attitude amongst respondents having dog ownership and those from urban locations was recorded. There is a need to aware rural area dwellers about the disease, its control and prevention.

## CONCLUSIONS

- The study revealed that yearly booster dose provided better results as compared to the serum samples from first time brought for vaccination and first booster category. So, owners which go for only one time vaccination for their dogs during their life span must be educated to yearly vaccinate their dogs.
- Sex-wise females showed lower antibodies as compared to males, which may be due to breeding season of bitches followed by immune suppression.
- The low specificity through I-ELISA (qualitatively) may also occur due to failure of maintenance of cold chain of vaccines affecting the efficacy of the vaccines being administered.
- Overall there was comparatively good approach towards Rabies knowledge and attitude amongst respondents with dog ownership and those living in urban location.
- Good practices prevailed in dog owners with high frequency of respondents showing positive responses.
- There is a need to aware rural area dwellers about the disease, its control and prevention.

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Vita

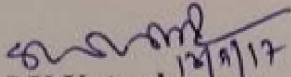
CERTIFICATE -IV

Certified that all the necessary corrections as suggested by the external examiner/evaluator and the Advisory Committee have been duly incorporated in the thesis entitled "Evaluation of Immunological response to rabies vaccination in Pet Dogs and KAP Assessment of their owners" submitted by Ms. Suhasani Tandon, Regd. No. J-15-MV-431

  
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