Indian J.L.Sci.2(1) : 139-142, 2012

Review

ANTI-RABIES ANTIBODIES

C K SINGH[a1], KARAN BANSAL[b], M DANDALE[c], AND PRANOTI SHARMA[d]

Department of Veterinary Pathology, Guru AngadDev Veterinary and Animal Sciences University, Ludhiana, India,

[a1]E-mail: rabiesck@gmail.com

[b]E-mail: karanbansal35@gmail.com

[c]E-mail: dr.mangesh9922@gmail.com

[d]E-mail: pranoti.sharma22@gmail.com

Rabies is an infectious disease characterized by an acute and profound dysfunction of the central nervous system caused by *Lyssa virus* of family *Rhabdoviridae*. Among all the viral diseases, rabies has been and is still regarded in all the parts of world as one of the most terrifying disease known to man. The incubation period for rabies is quite variable and on an average range from one to three months.

The established standard protocol for the diagnosis of the rabies involves collection and examination of brain tissues of the suspected animal after its death. It is, therefore, highly desirable to standardize diagnostic approaches that can be of help for establishing non-invasive ante-mortem diagnosis. One simple way could be to detect the antibody titre in the serum of animals suspected for rabies. Although a few workers have standardized laboratory techniques for detection of antibodies to rabies (Szkudlarek et al., 1982; Bhatia et al. 1984; Kavaklova et al., 1984; Boulos et al .,1987; Grassi et al .,1989; Jayakumar et al., 1995), but all have employed this approach only for analysis of immune response in vaccinated animals thereby also restricting such studies to the fixed, attenuated or inactivated strain of rabies virus.

Various techniques have been used for the detection of rabies antibodies in animals and human include Enzyme Linked Immuno Sorbent Assay (ELISA) (Perrin et al .,1986; Pixley, 1988; Grassi et al., 1989; Jayakumar and Ramdass 1991; Jayakumar et al .,1995,;Esterhuysen et al., 1995); RFFIT (Tepsumethanon et al., 1991, Mebatsion et al., 1992); Modified Rapid Fluorescent Focus Inhibition Test (RFFIT) (Aghomo et al., 1990; Brown and Rupprecht, 1990); Serum Neutralisation Test (Pixley 1988, Chauhan et al., 1991a); Haemagglutination Inhibition Test (Chauhan et al .,1991b); Modified Counter Immuno Electrophoresis (Chauhan et al., 1991a and Singh and Grewal ,1998b); SDS-PAGE (Pixley, (1988); Counter Immuno Electrophoresis (Diaz and Varela ,1977).

Attempts have been made to study the quantitative level of rabies antibodies. A micro-ELISA test was described for determining the rabies neutralizing antibody using the human diploid cell antirabies vaccine as the antigen. The protective titre >0.1 IU/ml was ascertained in all the 100 individuals who had a positive mouse neutralization test >1:10. None of the 250 negative serum samples could give E-492 values of >0.132 indicating thus the specificity of the test (Bhatia et al., 1984). In experimental rabies in sheep produced by street rabies virus of fox origin by intramuscular route, serum neutralization reactions rarely exceeded 0.1 IU/ml during lethal infection (Baltazar et al .,1986). The skunks those received inoculum containing 4x105 $MICLD_{50}$ had detectable serum neutralizing antibodies by 7-12 days after inoculation by intramuscular route. However, most of the skunks those received lower doses (40 to 4x103 $MICLD_{50}$) did not have detectable serum neutralizing antibodies until clinical signs began (Charlton et al., 1987).

Dogs and cats vaccinated with BPL inactivated fixed rabies virus received a booster after one year, challenged by a virulent strain of virus, showed antirabies titres higher than 0.1 IU/ml (Ganiere et al., 1989). Neutralizing antibodies in serum of 14 raccoon inoculated with street rabies virus were not linked to survival or mortality (Artois et al., 1989). Ferrets in three groups of five each received live vaccine made from Vnukovo-32 strain. One ferret each from two groups did not develop any antibodies, while in rest of the ferrets; the titres were ranged

[1]**Corresponding author**

from 1:11 to 1:25 (Matouch and Dousek, 1986).

Certain studies have been reported wherein antibody estimation has been attempted after challenge by street rabies virus. Twelve raccoons were inoculated with graded doses of street rabies virus. Out of these, raccoons that received the highest dose of inoculum, two raccoons developed serum neutralizing antibodies (Hill and Beran, 1992). Manika, (1994) carried out experiments aimed at proving the suitability of ferrets in the routine testing of rabies vaccine for human and animal use. A total of 40 ferrets received 4 vaccines, 2 for use in humans and 2 for use in animals. All animals developed antibodies of more than 0.5 IU/ml serum as determined by RFFIT. The antibody titre following injection of vaccine for animals was 4.1 IU/ml on day 28 and 5.86 IU/ml on day 56. The antibody titre following injection of the vaccines for humans was 6.64 IE/ml on day 28 and 0.87 IU/ml on day 56.

In another study, dogs were challenged intramuscularly twelve months after vaccination with 1 ml of suspension of a local street rabies virus strain containing 103.83 $MICLD_{50}$/0.03 ml. The mean titres were higher during two months after challenge (Jayakumar and Ramdass, 1991).

Singh and Grewal ,(1998b) detected rabies antibodies in experimentally infected buffalo calves by Modified Counter Immuno Electrophoresis. The buffalo calves were inoculated with 3 ml of infected mice brain suspension comprising of 376386 $MICLD_{50}$ of SRV via oral route and 1 ml of infected mice brain suspension comprising 125462 $MICLD_{50}$ of SRV via linear laceration. Sera samples were collected and antibody titre 1:8 was detected earliest at 5DPI and it increased to 1:64 by day 60.

Various workers have carried out comparison of different techniques employed for detection of antibodies against rabies virus.

Pixley, (1988) reported that dogs exposed to street rabies virus reacted to indirect ELISA as early as five days after exposure to street rabies virus and at 7 days after vaccination. ELISA was found to be more sensitive than RFFIT. In another study, rabbits vaccinated against rabies were challenged intracerebrally with 1.3 LD_{50} of virulent rabies virus and blood samples were collected on days 7, 14 and 21 days after inoculation. The antibody titre as tested by ELISA and SNT was more than 16 by day 21 (Nandi et al .,1990).

Diaz and Varela Diaz, (1977) employed Counter Immuno Electrophoresis to detect rabies antibodies and the results were compared with those obtained by Serum Neutralization Test. CIE was found to be even more sensitive than SNT.

Kavaklova et al., (1984) had employed ELISA and virus neutralization test for detection of rabies antibodies in human sera and found that double antibody ELISA was more sensitive and accurate technique for the quantification of rabies antibodies in human sera than virus neutralization test.

With testing of sera samples of dog, cat, fox, skunks and raccoon, Barton and Campbell, (1988) observed good correlation between results obtained with ELISA and that with fluorescence inhibition micro test. Mebatsion et al., (1992) developed an ELISA to detect IgG antibodies of rabies virus in sera samples of 270 foxes, 40 cats, 35 martens, 5 badgers and 4 polecats. In comparison with SNT close overall agreement was obtained.

In another study, the rabies antibodies were detected by MCIE, HAI tests and compared with conventional SNT as a yard stick. Both MCIE and HAI were sensitive and specific for the estimation of rabies antibodies. In general, MCIE and SNT showed insignificant difference (Chauhan et al., 1991b).

Modified Counter Immuno Electrophoresis was found to be as sensitive and specific for the estimation of rabies antibodies as SNT. Even the unitage obtained by the MCIE and SNT showed statistically insignificant differences and the correlation coefficients between the two methods was 0.697.

MCIE was found to be sensitive enough to detect a minimum level of antibodies (0.5 IU/ml) by using a 16 mA current per slide for 2 hours, indicator serum of 15 IU/ml and use of antigen at a concentration of 1:35. The test was found to be simple, quick and economical for titration of rabies antibodies (Chauhan et al., 1991a).

REFERENCES

Aghomo H.O., Ako-Nai A.K., Odouye O.O., Tomori O. and

Rupprecht C.E., 1990. Detection of rabies virus antibodies in fruit bats (*Eldolon helvum*) from Nigeria. J. Wild Life Dis., **26**: 258-61.

Artois M., Duchene M.J., Aubert M.F.A. and Blancou S., 1989. Experimental infection of the raccoon (*Procyon lotor*) introduced into Europe with fox rabies virus. Recuell de Medicine Veterinaire, **165**: 379-82.

Baltazar R.S., Aubert M.F.A., Blancou J., Ambert J., Barrat M.J., Chain E., Dardaine A., Patron C., Selve M. and Batlazar R.S., 1986. Experimental rabies in sheep susceptibility, symptoms, humoral immune response, lesion and virus excretion. Revue de Medicine Veterinaire ,**137** (12): 847-56.

Barton L.D. and Campbell J.B.,1988. Measurement of rabies specific antibodies in carnivores by an enzyme linked immunosorbent assay. J. Wild Life Dis. ,**24** (2): 246-58.

Bhatia R., Ray K., Anand B.R. and Rai Chaudhry A.N.; 1984. Detection of antirabies antibodies by enzyme linked immunosorbent assay. Indian J. Med. Res., **79**: 459-62.

Boulos A., Botros M., Thomas G., Ksiazek M.S.N., Scott and Fibbi A.K., 1987. Antibody response to Fermi antibodies vaccine in 21 patients bitten by rabid jackal. Transactions of the Royal Society of Trop. Med. Hyg., **81**: 381-82.

Brown C.L. and Rupprecht C.E., 1990. Vaccination of free ranging Pennsylvania raccoons (*Procyon lotor*) with inactivated rabies vaccine. J. Wild Life Dis., **26** (2): 253-54.

Charlton K.M., Casey G.A. and Campbell J.B., 1987. Experimental rabies in skunks immune response and salivary gland infection. Comp. Immunol. Microbiol. Infect. Dis., **10** (3/4): 227-35.

Chauhan A., Sood D.K., Saha S.M., Kapoor M., Aggarwal R.K. and Saxena S.N., 1991a. The quantitation of rabies specific antibodies. I. Modified counter immunoelectrophoresis a rapid and sensitive method. Biologicals, **19** (2): 93-95.

Chauhan A., Sood D.K., Saha S.M., Kapoor M., Aggarwal R.K. and Saxena S.N., 1991b. The quantitation of rabies specific antibodies. III. A comparative evaluation of modified counter immunoelectrophoresis, haemagglutination inhibition and serum neutralization titres of human sera. Biologicals.,**19** (2): 103-106.

Diaz A.M.O. and Varela Diaz V.M., 1977. The Counter Immuno Electrophoresis Test for detection of antibodies to rabies virus. Annales de Microbiologie,**128A** (3): 331-37.

Esterhuysen J.J., Prehaud C. and Thomson G.R., 1995. A liquid phase blocking ELISA for the detection of antibodies to rabies virus. J. Virol. Methods.,**51**: 31-42.

Ganiere J.P., Andre F.G., Blancou J., Artois M. and Aubert A.,1989. Vaccination of dogs and cats against rabies antibody titres and resistance to challenge two years after a booster injection of an inactivated vaccine with adjuvant. Revue de Medicine Veterinaire,**14** (4): 281-85.

Grassi M., Alexander R., Wandeler and Ernst P., 1989. Enzyme linked immunosorbent assay for determination of antibodies to the envelope glycoprotein of rabies virus. J. Clin. Microbiol., **27** (5): 899-902.

Hill R.E.Jr., Beran G.W. and Clark W.K., 1992. Demonstration of virus specific antibodies in the sera of free ranging Iowa raccoons (*Pryocyon lotor*). J. Wild Life Dis.,**28** (3): 377-385.

Jayakumar R., Chandramohan A., Govindrajan R. and Padmanaban V.D., 1995. Rapid dot enzyme immunoassay for the quantitation of rabies antibodies. Indian J. Pub. Hlth., **39** (1): 8-11.

Jayakumar R. and Ramdass P., 1991. Immunoglobulin response to rabies virus immunisation of dogs. Vaccine, **9** (9): 611-12.

Kavaklova L., Eskenazy M., Gancheva T. and Vacheva V., 1984. Enzyme linked immunosorbent assay for the quantification of rabies antibodies in human sera. Acta Virol. **28**: 422-27.

Manika C., 1994. The development of rabies antibodies in ferrets after injection of four different types of rabies vaccines. J. Vet. Med. Series B.,**41** (9): 574-79.

Matouch O. and Dousek J., 1986. Vaccinating ferrets (*Putorius furo*) against rabies with a cell culture vaccine. *Veterinaria medicina*, **31** (2): 123-28.

Mebatsion T., Sillero Z.C., Gottelli D. and Cox J.H., 1992. Detection of rabies antibody by ELISA and RFFIT in vaccinated dogs and in the endangered Simian jackal (*Canis simensis*) of Ethiopia. J. Vet. Med. Series B,. **39** (3): 233-35.

Nandi S., Goel A.G. and Pandey K.D., 1990. Immunogenic response to partially purified rabies virus as measured by ELISA. Indian J. Virol., **6** (1/2): 89-92.

Perrin P., Rollin P.E. and Sureau P., 1986. A rapid rabies enzyme immunodiagnosis (RREID) a useful and simple technique for the routine diagnosis of rabies. J. Biol. Stand.,**14**(3): 217-22.

Perrin P., Versmisse P. and Sureau P., 1988. A rabies agglutination test (RAT) for rabies antibody detection. J. Biol. Stand., **16**: 281-86.

Pixley C.E., 1988. An evaluation of an indirect enzyme linked immunosorbent assay and SDS PAGE, Western blot transfer techniques to detect rabies virus antibodies in canine sera. Dissertation Abstracts International B. Sci.Engg.,**49**(4): 962-63.

Singh C.K., Grewal G.S. and Bhardwaj M., 1998b. MCIE for quantitative analysis of antibodies to street rabies virus. Indian J. Anim. Sci., **68** (9): 925-27.

Szkudlarek L., Gut W., Jankowski M. and Serokowa D., 1982. Commercial antirabies vaccine used as antigen in a modified enzyme linked immunosorbent assay. Acta Virol., **26**: 98-101.

Tepsumethanon W., Polsuwan C., Lumlertdeachu B., Khawplod P., Hemachuda T., Chutivongse S., Wilde H., Chiewbarmrungkiat M. and Phanuphak P., 1991. Immune response to rabies vaccination in Thai dogs, a preliminary report. Vaccine.**,9** (9): 627-30.