Egypt, J. Anim. prod, **25**, No. 1 pp. 93-98 (1985)

Tracing of Rabies Virus "Flury Strain" in Embryonated Chicken Eggs.

Y.Z. Habashi, M. El-Bakry*; S.A. Salama--**

Depatment of Researches and Vaccines Production of Pit animals,

*** Veterinary Serum and Vaccines Research Institute,*

**** Departement of Microbiology and Parasitology, Faculty of Veterinary Medicine, Zagazig University. Egypt.*

THE CHICKEN embryo adapted low egg passage (L.E.P.) Flury strain of rabies virus was traced through fluorescent microscopy, in the blood and brains of chicken embryos inoculated with the virus via the egg yolk, The virus could be detected in the blood films on the third, fourth and fifth days post-inoculation, but brain smears showed that the virus began to appear from the fifth day to the tenth which is the time of chicken embryo harvestation for vaccine preparation.

Virus titration in mice indicated the presence of rabies virus in the blood, from the second day of inoculation up till the tenth day, but of low titre, ranging from 10^1 to $10^{2.4}$/0.03 ml. In case of brain samples, the virus titre was low on the second, third, and fourth day, being 10^1, $10^{1.75}$ and $10^{2.0}$/0.03 ml. respectively, then the virus began to propagate copiously from the fifth day up to the tenth day with a titre ranging from $10^{3.4}$ to $10^{4.8}$/0.03 ml.

Rabies constitutes a serious public health problem allover the world. Two theories had been established about the mode of rabies virus infection in the body of the victum. The first, which says, that rabies virus is transmitted via nerve tissues from the site of infected wound with saliva of a rabid animal, then wide spread till reached its C.N.S. as recorded by Goldwasser *et al.*, (1959) The second one, which suggests that the virus particles transmitted even at first via the blood streams before localization in the C.N.S., as previously described by Marie and urbain (1931). In addition, there is very little evidence that the blood stream is concerned with the dissemination of rabies virus following pripheral infection (Buxton, 1977)-.

Similarly Schweinburg (1932) could isolate both fixed and street virus from the blood of previously infected animals. In addition rabies virus could be isolated from ingorged ticks infesting infected rabbits with rabies virus (Remlinger and bailly, 1939).

From the previously mentioned openions, which each of them have their supporting facts, make us buzzeled, wheather these or that/or both are right. Many questions had been wared in our minds, specially from the common known knowledges on the clinical symptoms of the disease. Why then there is a severe headache, rapid rise of temperature which accompanied with great thirst of the bitten animal at the onset of the disease (Hagan and Bruner, 1961)?.

How the virus is excreated in the urine or with the milk of rabid lactating femals (Koradi, 1961 ; Hagan and Bruner, 1961; Buxton, 1977, respectively).

Moreover that attracked our attentions and encouraged us is that, how the virus is transmitted from the yolk sac to the embryos during preparation of the anti-rabies vaccine in our Laboratory. In such embryo's age, in which its nervous systems are not well developed, meanwhile, there is no way for transmission of all nutrients including the inoculated virus other than the ambliical viens surrounding the yolk sac.

Materials and Methods

Viruses : The low egg passage "Flury strain" of rabies virus and the standared challenge virus strain (C.V.S.), were furnished by pasteur Institute (Paris - France).

Buffer : Phosphate buffer saline (0.01 M), pH 7.6

Mice : White albino mice, bred locally, 3-4 weeks-old.

Chicken embryos : 7*th* days-old chicken embryos (Nicols bred) purchased from the General Poultry Company, were used.

Conjugate : A suitable commercial conjugate was obtained from Baltimore Biological Laboratories (U.S.A.), in the lyophilized form. It was diluted to 1:30, the dilution that gave good results without outo-fluorescence.

Conjugate diluent

Normal and infected mouse brain suspensions were used to serve as diluent and also as a control system. 20%normal mouse brain suspension and infected mouse brain with C.V.S.for diluting conjugate were prepared using as a diluent a 10% suspention of egg yolk, obtained from 7*th* days-old embryonated eggs in phosphate, buffer saline pH 7.6, the rest of the procedure was as recorded by Coons and Kaplan (1950).

Preparation of the materials

Groups of five living chicken egg embroys infected via yolk sac and normal controls, were randamly collected every 24 hr, begining from the 2*nd* till the tenth day post-inoculation, were used for the preparation of brain impression smears, as well as for virus titration in mice.

Brain impression smears

The procedure of this test was performed exactly as mentioned elsewhere (Laboratory technique in rabies, 1973). Positive control slides were prepared from brains of mice previously inoculated with C.V.S. and sacrified when moribund.

Blood films-

Blood pool was asceptically withdrawed from the amblical viens of each groups of the inoculated embryos. Blood films were prepared as mentioned in impression smears. Normal control blood films were also made from control embryos.

Direct Immunofluorescent staining

This test was performed qualitatively not quantitatively, in which blood films and brain impression smears under test were processed, with the normal specimens together with the positive control slides from the previously infected mice. Staining steps were done according to the technique of Coons and Kaplan (1950); Donald Dean (1966). Examination of the different prepared slides and films was carried out with Leitz-Laborlux fluorescence microscope using a dark-field condenser, 3mm. and 5 mm. BG_{12} filter, 100 X objective and 10 X occular.

Virus tracing

Five blood films and five impression smears of the test materials, prepared from different groups of chicken embryos, at different incubation periods, ranging from two up to 10 days, were stained as previously mentioned, examined by the fluorescent microscope and the results were recorded in the following table.

Virus titration in mice

Serial tenfold dilutions from the different specimens under test (blood and brains), were prepared for each as described in Laboratory technique in rabies (1973). The LD_{50} end point were calculated by the Reed and Muench method (1938), and then tabulated in the following Table.

Results

Virus titration in mice

(A) Blood : Virus titration of blood which had been taken every 24 hr. from the 2nd day post-inoculation till the 10th was relatively increased at the 3rd, 4th, and 5th days post-inoculation ($10^{2\cdot2}$, $10^{2\cdot4}$, $10^{2\cdot3}$ ED_{50}/ 0·03 ml. respectively), before and after these days the virus titre was low (from $10^{1\cdot6}$ to $10^{1\cdot0}$ ED_{50} / 0·03 ml.) as tabulated in the Table.

(B) Brains: As regards the virus titre in the brain, it is noticed that it was $10^{1\cdot75}$ and $10^{2\cdot0}$ ED_{50} / 0·03 ml. on the 3rd and 4th day post-inoculation, then start to propagate copiously after the fourth day as the titres were $10^{3\cdot4}$, $10^{4\cdot75}$ and $10^{4\cdot8}$ ED 50 / 0·03 ml. on the 5th, 7th, 9th and 10th day of inoculation respectively as shown in the Table.

Immunofluorescence tests

(A) Blood: As shown in the Table and figure, the virus could be traced in the blood films of inoculated chicken embryos from the 3rd up to the 5th day of inoculation, corresponding to the slightly increased in virus titre by the mice test, after which no more virus could by seen in the fluorescent's stained films. All attempts to obtain a speciefic immunofluorescence picture in the blood failed as the staine fade up rapidly.

(B) Brains: On the other hand, no virus could be detected in the brain impression smears of inoculated chicken embryos at the first four days after inoculation as the virus-titre is relatively low (4th day was suspecious), but

was clearly shown from the 5th day onwards corresponding to the increase in virus-titre, more obevious and numerous as the days progressed, as granular compact intracytoplasmic speciefic fluorescence shown in the figure.

TABLE 1. **Tracing of flury rabies strain in both brains and blood of chicken embryos.**

Results of Exam. after	Chicken embryo brain		Chicken embryo blood	
	Results of F.A.T.	Resuts of mice titre/0.03 ml.	Results of F.A.T.	Results of mice titre/0.03 ml.
2nd day	—ve	$10^{1.0}$	—ve	$10^{1.6}$
3rd day	—ve	$10^{1.75}$	+ve	$10^{2.26}$
4th day	+ve	$10^{2.0}$	+ve	$10^{2.4}$
5th day	+ve	$10^{3.4}$	+ve	$10^{2.31}$
7th day	++ve	$10^{4.75}$	—ve	$10^{1.4}$
9th day	+++ve	$10^{4.75}$	—ve	$10^{1.0}$
10th day	++++ve	$10^{4.80}$	—ve	$10^{1.0}$

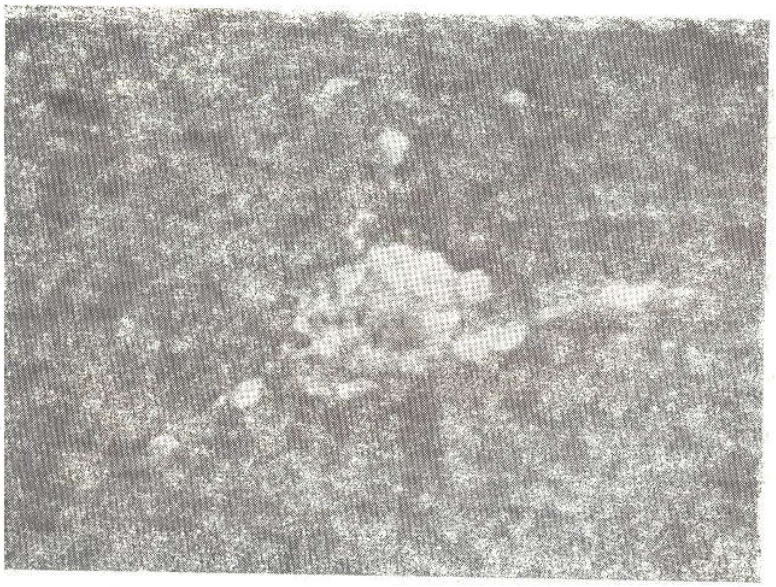

Fig. 1. Intracytoplasmic intensive accumulation of compact granular specific fluorescent-tagged antigen of rabies virus in the neuron of C.N.S. of infected mice intrcerebrally (*10th* day p.i) Mag. 1000 x.

Discussion

Fluorescent antibody technique considered one of the most rapid methods available for rabies diagnosis. In application of this test, we could detect the prescence of rabies viral particles for a certain period in the blood of infected chicken embryos, that agreeds with the finding of Marie and Urbain (1931), which could demonstrate fixed rabies virus in the blood of infected animals, as well as, support the foundations of Goldwasser *et al.*, (1959), who suggested that their is a viramic stage at the onsit of rabies disease. Occasionally, when severely lacerated wounds of the head and shoulders have been heavely infected, the virus may be carried to the brain via the blood stream (Medical Microbiology 1973).

Moreover, the isolation of street and fixed rabies virus from blood of previously infected animal (Schweinburg, 1932) or from ingorged ticks infesting infected rabbits (Remlinger and Bailly, 1939) or/ from milk and urine (Medical Microbiology, 1973).

In our present investigation, fixed rabies virus could be detected by F.A.T. in the blood at the 3rd, 4th and 5th days postinoculation from chicken embryos infected intra-yolk sacs, although at this time intervals, no virus could be traced from impression smears of similarly infected embryo' s brains. Contrary to that virus disappeared in blood films up to the 5*th* day onwards postinoculation. whereas, it could be demonstrated clear and copiously in brains of similarly infected embryos, corresponding to the increase of virus-titre measured by mice test (Koproski and Cox, 1948).

Our conclusions. From the results obtained in this research work, and from the allready mentioned references, that we suggest that there is a relatively short viramic stage, durate for a certain limitted period of time, mainly just after the exposure of infection, during which rabies virus is of a considerable titre in the blood stream, when this viramic period determined, perhaps the animal may be saved if hyperimmune anti-rabies serum is administered. Hoskins *et al.*, (1959), and Medical Microbiology (1973), mentioned that, specific prophylaxis by inducing passive protection administered by injecting hyperimmune horse serum (0·5 ml./ Kg. body weight) or/ concentrated immunoglobulin (at least 40 i.u.Kg body weight) and within 12 to 24 hr after the incident, protect the animal from the disease, but such serum is of no value after that period.

However, more research works must be undergo on this field in the future to compact such zoonotic fatal disease.

References

Buxton, A. (1977). *Animal Microbiology*, Vol. 2, P. 549.

Coons, A.H. and Kaplan M.H. (1950). Localization of antigen in tissue cells. II - Improvement in a method for the detection of antigen by means of fluorescent antibody. *J. Exp. Med.*, 91, 1—13.

Donald, J. Dean (1966). The fluorescent antibody test. Lab. Teh. in rabies. Wld. Health. Org *Monograph series.* 23, 2*nd* Ed., 62—127.

Goldwasser R.A., Kissling R.E., Caroki T.R. and Hosty T.S. (1959).Flourescent antibody staining of rabies virus antigens in the salivary gland of rabid animals. *Bull. Wld. Hlth. Org. 20,* 579.

Hagan, W.A. and Bruner D.W. (1961). The *Infectious Disease of Domestic Animal*", 4*th* Ed., Comstock Publishing Associates division of Cornell Univ., IThaca, N.Y., 775—778.

Hoskins, H.P., Lacroix, J.V. and Mayer K. (1959).Canine Medicine, 2*nd* Ed., Am. Vet. Publications, Inc., Santa Barbara, California, P. 694.

Konradi (1961) *The Infectious Disease of Domestic Animals,* 4*th* Ed., Comstock publishing associates adivision of Conell UNIV IThaca N,Y., P. 778.

Koprowski, H. and Cox, H.R. (1948).Studies on chicken embryo adapted rabies virus : I — Culture characterisation and pathogenicity. *J. Immunol.*. 60, 533—554.

Laboratory Technique in Rabies (1973). 2*na* Ed. Wld. Halth. Org. *Monograph Series* No. 23, Geneva, P 27 - 33 and 106 — 107.

Marie, A.C., and A. Urbain (1931). Virus rabique Fixe et Virulence du sang compt rend. *Soc. de Biol.* 106. 166

Medical Microbiology . (A guide to the Laboratory diagnosis and control of infection) 1973 12*th* Ed. Vol. 1, Microbial infections. The English language book society and Churchill, Livingstone, Edinburgh London and New York, P. 499, 501.

Romlinger P., and Bailly J. (1939) Development possible du virus rabique dans l'organism de la tique du chien (Rhipice phalus sanguineus). *Ann Inst Pasteur.* 62, 436.

Reed, L.J., and Muench A. (1938). A simple method for estimating fifty percent end points *Am J. Hyg.*, 27, Vol. 493.

Schweinburg, P. (1932).Uber den Nachweis von Lyssa Virus in verschiedenen Orga nen. *Zbl. Bakt. Abt.* I., 123, 434—448.

تتبع فيروس مرض الكلب عترة (فلورى) فى اجنة الدجاج

يوسف زكى ، محمد البكرى وسيد سلامة

يهدف هذا البحث تتبع فيروس مرض الكلب عترة (فلورى) فى اجنة الدجاج ـ لاستبيان الطريق الذى سيسلكه هذا الفيروس من مكان حقنه فى كيس المح وحتى تمركزه فى الجنين (عن طريق الاوعية الدموية أو عن طريق الاعصاب المحيطة به) ـ وقد استخدم فى هذا البحث الكشف على الفيروس فى عينات اقلام الدم من الاوعية الدموية المحيطة بكيس المح وشرائح امخاخ أجنة الدجاج فى أوقات متفاوتة باستخدام الميكرسكوب المشع مع مقارنته باختبار الحماية فى الفئران السويسرية ٠

وقد أمكن الحصول على الفيروس بواسطة الاجسام المناعية المشعة فى اقلام الدم من اليوم الثالث والرابع والخامس من الحقن ، فى حين انه بدأ فى الظهور فى شرائح امخاخ اجنة الدجاج من اليوم الخامس وحتى اليوم العاشر من الحقن وهو يوم جمع الاجنة فى تحضير لقاح الكلب ٠

وباستخدام اختبار المعايرة فى الفئران أثبت ظهور فيروس مرض الكلب فى شرائح الدم من اليوم الثانى وحتى اليوم العاشر ولكن بقوة ضعيفة ١ ٣٫٤

نسبيا ـ تتراوح مابين ١٠ الى ١٠ ، اما فى حالة شرائح امخاخ اجنة الدجاج فظهر به الفيروس بصورة مكثفة من اليوم الخامس حتى اليوم العاشر ٣٫٤ ٤٫٨

بقوة عيارية عالية تتراوح بين ١٠ الى ١٠ / ٠٫٠٣ مليمتر ٠