

## RPR Test for Serological Survey of Rabbit Syphilis in Companion Rabbits

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ABSTRACT. Since the RPR (rapid plasma regain) test was found to be useful for the diagnosis of rabbit syphilis, serological survey by this test has been carried out in Japanese companion rabbits. A hundred virgin household rabbits kept alone and without signs and history of syphilis were examined by RPR test from April 2001 to March 2002, in Tokyo, Japan. The test was positive in 35 cases and negative in 65 cases. RPR negative rabbits should be selected for breeding to prevent the spread of rabbit syphilis in companion rabbits in Japan.

KEY WORDS: rabbit syphilis, RPR test, *Treponema*.

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The rapid plasma reagin (RPR) test, a method for detecting antibodies against a lipid antigen, is one of the serological tests for syphilis, a human venereal disease caused by *Treponema pallidum*. Rabbit syphilis is a rabbit venereal disease caused by *Treponema paraluis-cuniculi*. This spirochete is transmitted by direct contact, especially during mating, and from a doe to kits [5-8]. Though several serological methods have been used to detect rabbit syphilis including the RPR test, VDRL test, FTA-ABS test, and Wassermann reaction [1, 3, 4, 6, 8], the RPR test was used in this study because it has been reported to be a specific [3] and useful diagnostic test for rabbit syphilis [3, 4].

Suspected cases of rabbit syphilis presented in the authors' clinic since 1999. The pathogenic organism, however, was not easy to detect by darkfield microscopy of skin scrapes, and pathological examination of biopsy specimens could not be carried out for the patient rabbits. Therefore, these cases were tentatively diagnosed from clinical signs and response to chemotherapy, and then confirmed by the RPR test. In a previous report, all 16 cases diagnosed clinically as rabbit syphilis showed a positive reaction in the RPR test [11]. All 16 cases were thought to be due to maternal infection, since none of these rabbits had mated. On the bases of this finding, the test subjects in this study were limited to individuals that had neither mating experience nor direct contact with other rabbits. This serological survey was performed to detect sero-positive rabbits without clinical signs, in order to eradicate this disease from companion rabbits by selective breeding.

Approximately three thousand household companion rabbits visited Saito Rabbit Clinic (Kita-Ku, Tokyo) from April 2001 to March 2002. Among them, 100 virgin household rabbits (58 male and 42 female), with no contact with other rabbits and no signs or history of syphilis, were examined by the RPR test. Their age ranged from 3 months to 9 years and 7 months (average: 3 years and 10 months). Blood samples were taken from the intermedial branch of the caudal auricular artery or cephalic vein, and 0.05 ml of heparinized whole blood was used for analysis. Test kits "RPR test Kokusai" (International Reagents Corporation, Kobe,

Japan) were used in this study.

RPR testing of 100 rabbits produced a positive result in 35 cases and a negative result in 65 cases. The rate of positive results was not significantly different between males and females ( $p=0.4712$ ,  $t=0.2356$ , by  $t$ -test) (Table 1). To find out the influence of aging, the RPR positive rate in each age was checked and is shown in Fig. 1. No correlation between age and the rate of positive results was detected. Rabbits testing positive showed no distinctive features or tendencies.

During this investigation period, three cases with suspected skin lesions were determined not to be syphilis, following tentative therapy. The lesions in these cases resembled rabbit syphilis, but they did not improve after administration of chloramphenicol (Pediatric Chloromycetin Palmitate, Sankyo, Tokyo, Japan) (55 mg/kg BID p.o.) for a week. One rabbit had lesions on its eyelids, one on its auricles and eyelids, and one on its genitalia and anus. These three rabbits were also tested by RPR, indicating one positive and two negative results. The profiles, symptoms, and results of the RPR tests in these three cases are shown in Table 2. Lesions can be frequently observed on the genitalia, nose, lips, and eyelids [5, 6, 9], requiring a thorough differential diagnosis [6, 7, 9]. The RPR test can be useful in such cases to support presumptive diagnoses based on clinical signs, and could be helpful in the differential diagnosis of dermatophytosis, pyoderma, and trauma.

The RPR test is one of the serological tests available for

Table 1. Results of RPR test in 100 rabbits without symptoms

|               | Male | Female | Total |
|---------------|------|--------|-------|
| Positive*     | 22   | 13     | 35    |
| Negative*     | 36   | 29     | 65    |
| Total*        | 58   | 42     | 100   |
| Positive rate | 38%  | 31%    | 35%   |

\* The numbers showed are the actual numbers of cases.

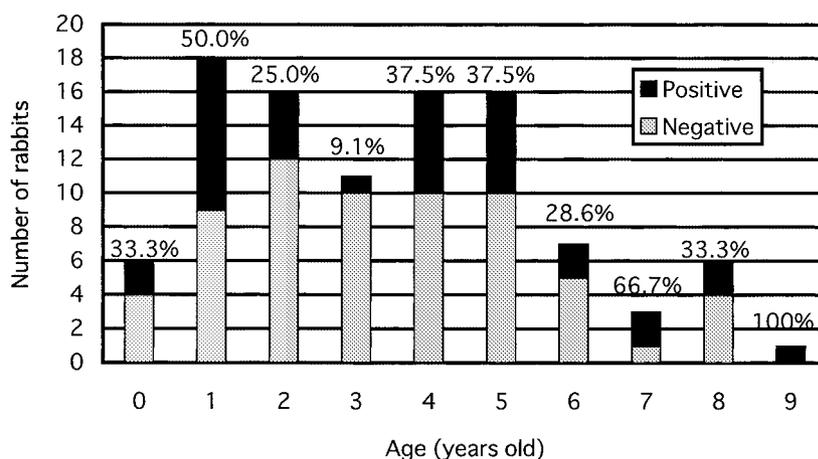


Fig. 1. The RPR positive rate in each age group. Although there was no correlation between age and positive rate, the rate was low in three year olds and high in one year olds. This disease may have been present for a long time in Japan, because 4 and 5 year old rabbits showed a rather high rate. Furthermore, there were seven positive results in old rabbits (6 to 9 years old).

Table 2. Syphilis-like, but non-syphilis, cases in rabbits

| Case No. | Profile |                   |     | Symptom   | Response to chemotherapy | RPR test |
|----------|---------|-------------------|-----|---|--------------------------|----------|
|          | Sex     | Breed             | Age |   |                          |          |
| 1        | Female  | ML <sup>a)</sup>  | 4mo | Dermatitis on eyelids   | Not reacted              | -        |
| 2        | Male    | AFL <sup>b)</sup> | 5mo | Scab on inside of auricles and medial angles of eyelids, and sneeze | Not reacted              | +        |
| 3        | Female  | AFL               | 8mo | Redness, swelling and scab on genitalia and anus                    | Not reacted              | -        |

a) ML: Mini-Lop

b) AFL: American Fuzzy Lop.

human syphilis, and has also been found to be useful for diagnosis of rabbit syphilis [1-3]. In a previous report [11], we showed that all 16 cases diagnosed clinically as rabbit syphilis tested positive by RPR, suggesting that the RPR test might agree well with a diagnosis based on clinical signs and response to chemotherapy. Although the possibility of a pseudo reaction from chylemia cannot be ruled out, the RPR test has proven to have a higher specificity than the VDRL test in detecting *Treponema parvulus-cuniculi* infection [3]. An RPR negative result does not necessarily indicate the absence of any antibody, because some reports have described rabbits with a negative RPR test, which test positive by VDRL or FTA-ABS [3, 4]. In fact, the RPR test is less sensitive than either the VDRL or FTA-ABS tests [3]. Conversely, an RPR positive result does not necessarily indicate that the animal is suffering from rabbit syphilis, as supported by the results of this study. Thirty five % of healthy rabbits showed a positive reaction, and one of three cases ruled not to be syphilis, based on lack of response to chemotherapy, showed a positive reaction. Based on these considerations, the RPR test is sufficiently sensitive and specific for clinical use. In addition, this kit is easily

obtained and used in animal hospitals.

Since the 35 rabbits testing positive did not have any possible exposure aside from their mothers, they could be positive due to maternal infection. However, it is uncertain whether they are self-cured permanent carriers. Further study of mothers and their offspring may be necessary.

Although there was no correlation between age and rate of positive results, the rate was low in three year olds (9.1%) and high in one year olds (50%) (Fig. 1). This disease seems to have been present for a long time in Japan, as 4 and 5 year old rabbits showed a rather high rate of positive test results (37.5%), and there were also seven positive cases in old rabbits (6 to 9 years old).

Since these old rabbits were confirmed to have neither mated nor been in contact with other rabbits, they might have become positive by maternal infection. If true, a positive reaction has persisted for a very long time. In cases of human syphilis caused by *T. pallidum*, RPR antibody titers become negative several months to years after treatment [2, 10]. In rabbit syphilis caused by *T. parvulus-cuniculi*, seroconversion to negative titers was also reported 2 to 4 months after treatment, although many rabbits remained positive

more than a year later [4].

Since many pet rabbits presently for sale in Japan come from breeders in this country, many breeding does and bucks may be infected with this spirochete. The occurrence of rabbit syphilis in companion rabbits will continue unless selection of breeding rabbits is carefully considered. It may be important to perform an RPR test before breeding, in order to prevent the spread of infection. On the other hand, this disease is known to cause abortion, stillbirth, and metritis in breeding does [5–7]. Therefore, infection of breeding rabbits can cause decreased fertility and productivity, as well as maternal infection.

In conclusion, if used for selective breeding, the RPR test could be a means to prevent the spread of *Treponema paraluis-cunicul* infection, as well as a supportive method for diagnosis of this disease.

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