Veterinary Immunology and Immunopathology, 7 (1984) 245—254
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

HUMORAL IMMUNE RESPONSE OF DOGS AFTER VACCINATION AGAINST LEPTOSPIROSIS MEASURED BY AN IgM- AND IgG-SPECIFIC ELISA

E.G. HARTMAN[1], M. VAN HOUTEN[1], J.F. FRIK[1] and J.A. VAN DER DONK[2]

[1]Department of Bacteriology, Veterinary Faculty, University of Utrecht, P.O. Box 80 171, 3508 TD Utrecht (The Netherlands)

[2]Department of Immunology, Veterinary Faculty, University of Utrecht. Present address: Laboratory of Histology and Cell Biology, Medical Faculty, University of Utrecht, Nic. Beetsstraat 22, 3511 HG Utrecht (The Netherlands)

(Accepted 9 March 1984)

ABSTRACT

Hartman, E.G., van Houten, M., Frik, J.F. and van der Donk, J.A., 1984. Humoral immune response of dogs after vaccination against leptospirosis measured by an IgM- and IgG-specific ELISA. Vet. Immunol. Immunopathol., 7: 245-254.

An IgM- and IgG-specific ELISA was used to measure the antibody response stimulated in dogs by vaccination with a leptospiral bacterin containing chemically inactivated Leptospira interrogans serotype icterohaemorrhagiae and serotype canicola leptospires. All dogs produced anti-leptospiral IgM and IgG. The IgM production was of the primary response type after each vaccination (primary vaccination, booster vaccination and annual revaccination). A substantial anti-leptospiral IgG response could be demonstrated only after the first booster vaccination and the annual revaccination. Annual revaccination resulted in a higher and much longer persisting IgG response than did the first booster vaccination. A revision of the vaccination scheme is suggested.

INTRODUCTION

Canine leptospirosis is distributed world-wide and generally associated with infection by Leptospira interrogans, serotype icterohaemorrhagiae and canicola (Michna, 1970). In the Netherlands infections with serotype canicola are restricted to the canine population, whereas serotype icterohaemorrhagiae is spread by the brown rat as it is world-wide (Hartman, 1977).

The first successful vaccination experiments in dogs were carried out by Dalling and Okell (1926) with a killed serotype icterohaemorrhagiae vaccine and by Ottosen (1946) with a serotype canicola vaccine. Prevention of leptospirosis in dogs through vaccination with a chemically inactivated bacterin of mixed cultures of the above-mentioned serotypes is widely practiced nowadays. Before these bacterins are licenced they must pass a potency test in hamsters as prescribed in the U.S. Code of Federal Regulations (Standard Requirements B-41) and the European Pharmacopoeia. This hamster protection test, however, does not provide information concerning the degree and duration of protection against clinical leptospirosis obtained through vaccination of dogs. The hamsters are challenged only during the 3rd week after vaccination.

0165-2427/84/$03.00

The degree of immunity conferred by vaccination is not related to the agglutination titre. Thus, an animal may be protected for a considerable period after serum agglutinins have declined below detectable levels. Leptospiral bacterins often induce protection without at all stimulating significant levels of agglutinating antibodies. Using the agglutination reaction as the sole measure of immuno-protection, one could falsely conclude that the bacterins had failed to be sufficiently immunogenic (Heath and Box, 1965; Hanson et al., 1972; Hanson, 1973) yet, the discrepancy between agglutination and protection is a result of the different properties of the various classes of immunoglobulins produced after antigenic stimulation through vaccination. IgM antibodies are the first to be detected after antigenic stimulation and can be seen as a primary response directly proportionate to the antigen concentration for relatively short periods following each exposure to the same (inactivated) antigen. IgG antibodies can be detected somewhat later after primary antigenic stimulation and respond anamnestically following secondary exposures (Pike, 1967; Hood et al., 1978). Due to their high valency IgM antibodies are highly effective agglutinators, whereas IgG antibodies are more closely associated with neutralizing activities and phagocytosis and only partially contribute to the agglutination reaction (Hood et al., 1978). From the above-mentioned one can conclude that the degree and duration of the production of specific anti-leptospiral IgG is the most important criterion to determine the quality of (humoral) immunity after vaccination.

The aim of the present investigation was to determine the production of specific anti-leptospiral IgM and IgG after vaccination of dogs against leptospirosis using the ELISA technique, which has been shown to be a very useful tool for this purpose (Hartman et al., 1984).

MATERIALS AND METHODS

Dogs

Primary vaccination. Eight purebred beagles (six females 7 to 9 months of age and two males 18 months of age) were vaccinated subcutaneously on the thoracic wall with one recommended dose of a commercial bacterin. The bacterin consisted of a chemically inactivated whole culture mixture of *Leptospira interrogans* serotypes icterohaemorrhagiae and canicola. The vaccination was repeated three weeks later.

Annual revaccination. Seven purebred beagles (all females circa 2½ years of age), which had been vaccinated one and two years before, were subcutaneously vaccinated for the third time with a single recommended dose of the same commercial bacterin. All dogs were vaccinated with bacterin of the same lot.

Serum sampling. Serial serum samples were collected from each dog before and after vaccination and stored at -70°C until use.

Microscopic aglutination test (MAT)

The MAT (Turner, 1968) was carried out in Cooke Microtiter plates. Leptospires were cultivated in a polysorbate-80 bovine albumin medium (Difco). Cultures of Leptospira interrogans serotypes icterohaemorrhagiae strain Kantorowics and canicola strain Hond Utrecht IV were used as antigen (density approximately 10^8 leptospires per ml). Serial two-fold dilutions of serum starting with 1 in 20 were made in Chang buffer (Chang, 1947). The titre is expressed as the reciprocal of the highest dilution of serum at which at least 50% of the leptospires were agglutinated (WHO, 1967).

ELISA

The ELISA was carried out in microtitre plates (Cooke Dynatech, Microelisa) coated with the outer envelope antigen of Leptospira interrogans serotypes icterohaemorrhagiae strain Kantorowics and canicola strain Hond Utrecht IV. In consecutive steps (1) two-fold diluted dog serum,(2a) rabbit anti-canine IgM (μ chain specific) or (2b) rabbit anti-canine IgG (γ chain specific), then (3) goat anti-rabbit IgG (H+L) horse-radish peroxidase conjugate (Miles Laboratories) and finally (4) substrate (ortho-phenylenediamin) were added. After each incubation step the plates were thoroughly washed with tapwater-Tween-20. The test was read on a Dynatech Microelisa Minireader MR 590 (Pope et al., 1982; Hartman et al., 1984).

Data analysis

All antibody titres are expressed as the arithmetic mean of the log10 of the reciprocal titres and presented as a linear function of time in weeks (Figures 1-4).

RESULTS

Primary vaccination

Serological examination of the 8 beagles before the primary vaccination experiment demonstrated no agglutination titres equal to or exceeding 20; ELISA IgG titres were <20 and ELISA IgM titres were $\leqslant 40$ in all dogs and should be considered to be negative (Hartman et al., 1984). The antibody response of the primary vaccinated beagles is shown in the Figures 1 and 2.

IgG response. Primary vaccination did not stimulate a substantial production of IgG to either serotype icterohaemorrhagiae or serotype canicola during the three weeks preceding the booster vaccination. In only two dogs a titre of 40 was demonstrated to serotype canicola and in one dog to serotype icterohaemorrhagiae. After the first booster vaccination a sharp rise of the IgG titre was demonstrated: an eleven-fold rise of the mean anti-canicola IgG titre and an eight-fold rise of the mean anti-icterohaemorrhagiae IgG titre. The maximum

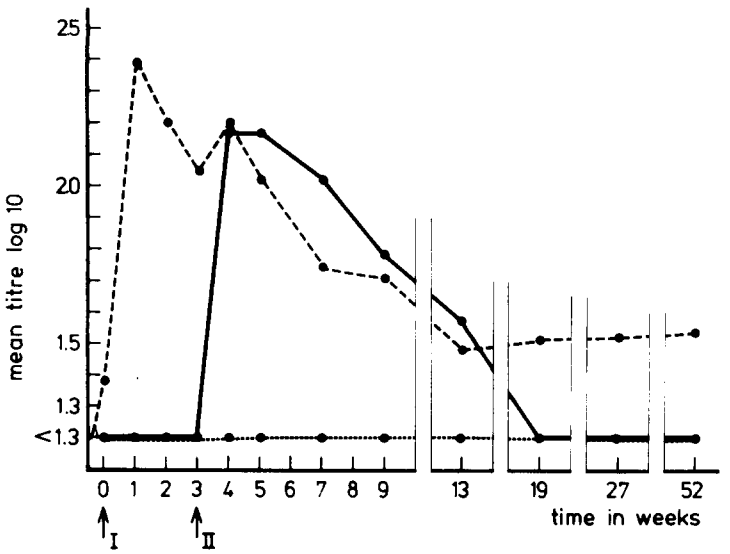

Fig. 1. Antibody response to the canicola component of a bivalent bacterin as measured by the MAT and the IgM-, resp. IgG-specific ELISA in eight dogs after primary vaccination: first vaccination (I) and booster vaccination (II).
----IgM; ——IgG; ·····MAT.

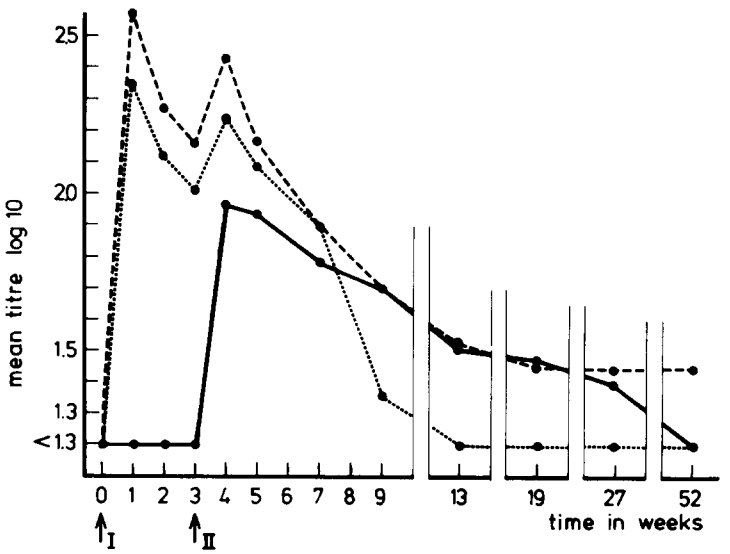

Fig. 2. Antibody response to the icterohaemorrhagiae component of a bivalent bacterin as measured by the MAT and the IgM-, resp. IgG-specific ELISA in eight dogs after primary vaccination: first vaccination (I) and booster vaccination (II).
----IgM; ——IgG; ·····MAT.

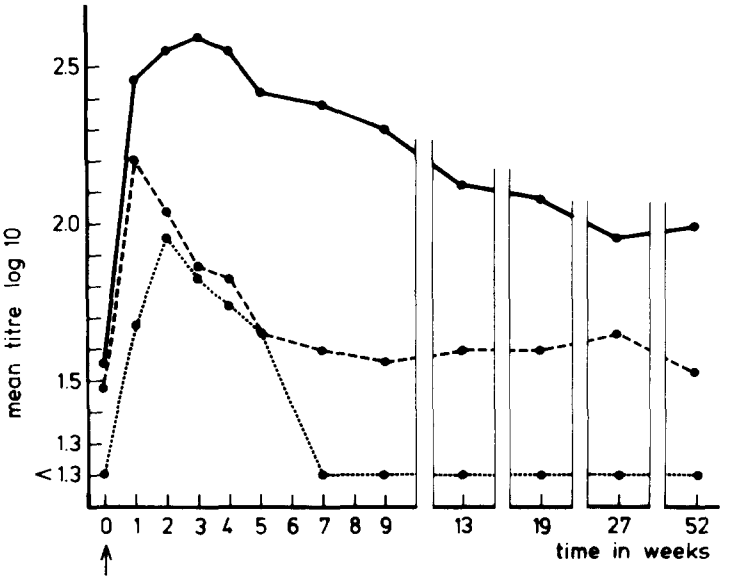

Fig. 3. Antibody response to the canicola component of a bivalent bacterin as measured by the MAT and the IgM-, resp. IgG-specific ELISA in seven dogs after the 2nd annual revaccination (arrow).
----IgM; ——IgG; ·——— MAT.

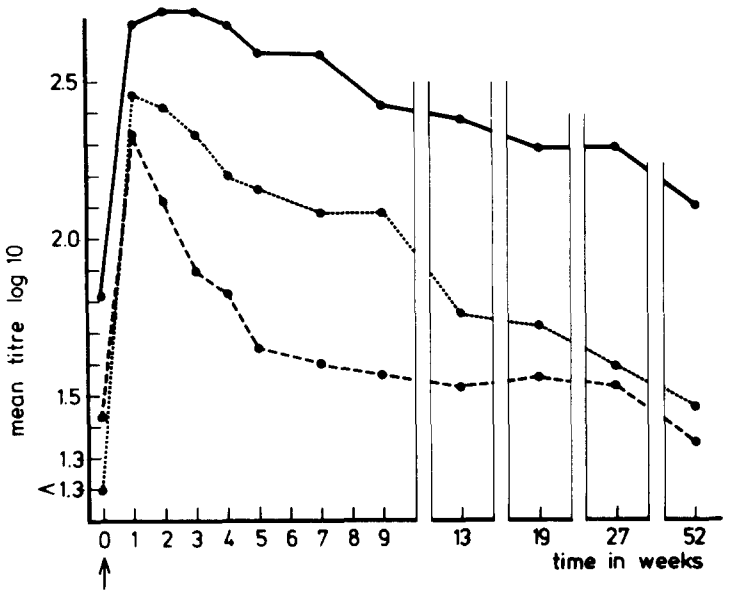

Fig. 4. Antibody response to the icterohaemorrhagiae component of a bivalent bacterin as measured by the MAT and the IgM-, resp. IgG-specific ELISA in seven dogs after the 2nd annual revaccination (arrow).
·----IgM, ——IgG; ——— MAT.

of the mean IgG titre to both serotypes was attained one week after the booster vaccination. Thereafter the mean IgG titre to both serotypes gradually declined to reach the lowest detection level 1 in 20 (1.3) at 4½ months and 6 months post-vaccination, respectively for serotypes canicola and icterohaemorrhagiae; however, the 1 in 40 (1.6) level (one dilution step above the lowest detection level) was already reached within 10 weeks after the booster vaccination (both serotypes).

The immune response of the individual dogs varied to some extent. The maximal IgG titre varied from 40 to 320 (icterohaemorrhagiae) and from 80 to 320 (canicola). In two dogs ("low responders") the IgG titre declined to 20 within three weeks (icterohaemorrhagiae) and seven weeks (canicola) following the booster vaccination. After half a year in only two dogs an IgG titre of 80 could still be demonstrated to both serotypes. One year after the primary vaccination all dogs had IgG titres ≤ 40.

IgM response. The IgM response to both serotypes was of the primary response type after the first and booster vaccination. The mean IgM titre to both serotypes attained its maximum one week after the first vaccination (varying from 160 to 640 to both serotypes) and also one week after the booster vaccination (varying from 80 to 320 and 640 respectively to canicola and icterohaemorrhagiae) and declined below the 1 in 40 level within 10 weeks after the booster vaccination.

MAT response. The mean MAT titre pattern with respect to serotype icterohaemorrohagiae closely followed the mean IgM pattern and declined to the lowest detection level (1 in 20) within 10 weeks after the booster vaccination. The mean MAT titre to serotype canicola did not exceed the lowest detection level for one year following primary vaccination. In only two dogs an anti-canicola MAT titre >20 (80 to 160) was detected during the first two weeks after the first and booster vaccination.

Annual revaccination

The antibody response of the annually revaccinated beagles is shown in the Figures 3 and 4.

IgG response. Revaccination of the beagles, which had been vaccinated annually two times before, resulted in an anamnestic rise of the IgG titre to both serotype icterohaemorrhagiae and canicola to attain a maximum 2 to 3 weeks after the revaccination. In the individual dogs the maximum varied from 320 to 2560 to both serotypes, with the exception of one dog (probably a "low responder") which demonstrated a maximal IgG titre of only 80 to serotype icterohaemorrhagiae and 40 to serotype canicola. Although the mean IgG titre to both serotypes declined gradually after attaining the maximum, it remained at a higher level than before the revaccination for at least one year. However, in

two dogs including the "low responder", the anti-canicola IgG titre decreased to ≤40 by the end of the year following the revaccination.

IgM response. The IgM response to both serotypes was of the primary response type as it was after the first and booster vaccination of the primary vaccinated dogs. The mean IgM titre attained its maximum also one week after the vaccination. The maximal IgM titre varied from 80 to 320 with respect to both serotypes. The mean IgM titre to both serotypes declined to a negative IgM level (≤40) within seven weeks after the revaccination. The low IgG responder showed a "normal" IgM response.

MAT response. The MAT response to serotype canicola closely resembled the IgM response to the same serotype attaining a maximum two weeks after the revaccination. The mean MAT titre declined to below the lowest detection level within six weeks. The MAT response to serotype icterohaemorrhagiae was more pronounced, reached a maximum one week after the revaccination and only gradually declined thereafter. It parallelled the IgG response to serotype icterohaemorrhagiae to a certain extent. The maximal MAT titre to serotype icterohaemorrhagiae varied from 160 to 640, whereas the maximal MAT titre to serotype canicola varied from 20 (the same "low responder") to 160.

DISCUSSION

The immune responses to leptospiral bacterins have been difficult to evaluate using the MAT because the majority of the vaccinated animals develop either a low agglutination titre or fail to develop any agglutination titre at all following vaccination. However, results of hamster protection test and challenge studies show that protective antibodies do develop after vaccination and persist after the agglutination titre has disappeared (Heath and Box, 1965; Negi et al., 1971; Huhn et al., 1975).

Immunoglobulin G is the principal class of antibody for protection, whereas the IgM class of antibody is primarily associated with agglutination (Hanson, 1973). Negi et al. (1971), using the hamster passive-protection test, demonstrated that protective antibodies were present in both the IgM and IgG fraction of calf serum, being more prevalent, however, in the IgG fraction. Hamster passive-protection tests are laborious and expensive and not suited for routine testing of the humoral immune response after vaccination. Although Huhn et al. (1975) have demonstrated the relation between the resistance induced in vaccinated dogs and that induced in vaccinated hamsters by a challenge two weeks after vaccination and Desmecht (1982) found that hamsters were protected for at least 18 months after two vaccinations three weeks apart with 1/10 of the dose of a vaccine for dogs, hamster protection tests as prescribed in the U.S. Code of Federal Regulations and the European Pharmacopoeia in principle provide no information concerning the duration of immunity in dogs.

A simple reproducible serological test to measure the IgG response would facilitate the evaluation of the humoral immune response after vaccination. Tripathy et al. (1973) described the use of the growth-inhibition test to detect antibody to leptospires in cattle vaccinated with leptospiral bacterins and found that the growth-inhibiting activity was largely associated with the IgG antibodies. The major disadvantage of this test is that an incubation period of approximately two weeks is required before results can be read. Using our ELISA technique the results can be read within five hours. The results presented by Bey and Johnson (1978, 1983b), using the microscopic agglutination test and the leptospiricidal assay to monitor the humoral immune response of dogs after vaccination, were partially inconsistent with the results described in the present paper. They found that both IgM and IgG were produced in similar amounts in vaccinated dogs over an observation period of two years. In our experiment, using the ELISA technique, anti-leptospiral IgM could only be detected for short periods of time following each vaccination, whereas a substantial production of anti-leptospiral IgG was not detected before one week after the first booster vaccination; the IgG titres being far more pronounced and of a much longer duration after the annual revaccination. The fact that the IgG response more or less parallelled the IgM response after the first booster vaccination is in agreement with the results of Bey and Johnson (1978, 1983b). However, IgM titres cannot quantitatively be compared with IgG titres due to different avidities of both immunoglobulins.

As protection is primarily associated with IgG antibodies and as anti-leptospiral IgG titres of maximal 80 could only be demonstrated for a limited number of weeks after a single vaccination, as was demonstrated in four other dogs, primary vaccination of dogs against leptospirosis should include a booster vaccination. The rapid decline of the IgG titre after the first booster (Figures 1 and 2) might result in a too low level of humoral immunity and an increased risk of infection. Revaccination before the end of the 3rd month after the first booster could probably prevent this risk of infection. Annual revaccination, resulting in the production of relatively high anti-leptospiral IgG titres persisting for at least one year in the majority of the dogs, as shown in Figures 3 and 4, seems satisfactory.

As described for other bacterins (Hanson, 1973), the canicola component of the bacterin, used in this experiment, did not stimulate a detectable MAT titre ($\geqslant$20) in the majority of the dogs after the primary vaccination (+ booster), whereas the icterohaemorrhagiae component stimulated a MAT response similar to the IgM response to that serotype as did the canicola component in the group annually revaccinated beagles (Figure 3). Agglutination titres to serotype canicola were considerably lower than agglutination titres to serotype icterohaemorrhagiae. Live serotype canicola antigen apparently cannot easily be

trapped by agglutinating antibodies (IgM) in spite of relatively high anti-leptospiral IgM titres (Figure 1) as detected by the ELISA using the outer envelope antigen.

A certain amount of biological variation, resulting in varying antibody responses to vaccination, was demonstrated in the group of beagles used in the present vaccination experiment. This variation could probably be masked when larger amounts of antigen were used in the vaccine (Huhn et al., 1975).

CONCLUSIONS

The ELISA technique detecting specific anti-leptospiral IgM and IgG proved to be a very suitable test to measure the humoral immune response in dogs after vaccination with a chemically inactivated leptospiral bacterin.

Vaccination titres do not disturb the serodiagnosis of acute leptospirosis because, as a rule, dogs suffering from acute leptospirosis demonstrate a relatively high anti-leptospiral IgM titre together with a low or negative anti-leptospiral IgG titre (Hartman et al., 1984). Such titres were found only during the time between the first and the booster vaccination (in primarily vaccinated dogs).

The results of the present investigation suggest the necessity of a revision of the vaccination scheme. Addition of an adjuvant to the leptospiral bacterins could perhaps improve the degree and duration of the immune response as was demonstrated by Bey and Johnson (1982a). However, as the primary vaccination experiment was carried out in older dogs, the results of the present experiment are not necessarily applicable to puppies of circa three months of age.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Mrs. G.M.S. van Dam-Knubben for excellent technical assistance. Prof.Dr. W.A. de Voogd van der Straaten for statistical assistance and Drs. S.H. Loeffler for linguistic advice. The staff of the Department of Small Animal Obstetrics and Gynecology, Veterinary Faculty, University of Utrecht (head Prof.Dr. C.H.W. de Bois) is gratefully acknowledged for the permission to use the beagle colony for the vaccination experiment.

REFERENCES

Bey, R.F. and Johnson, R.C., 1978. Humoral immune response of dogs vaccinated with leptospiral pentavalent outer envelope and whole culture vaccines. Am.J.Vet.Res., 39: 831-836.

Bey, R.F. and Johnson, R.C., 1982a. Leptospiral vaccines in dogs: Immunogenicity of whole cell and outer envelope vaccines prepared in protein-free medium. Am.J.Vet.Res., 43: 831-834.

Bey, R.F. and Johnson, R.C., 1982b. Immunogenicity and humoral and cell-mediated immune responses to leptospiral whole cell, outer envelope, and protoplasma cylinder vaccines in hamsters and dogs. Am.J.Vet.Res., 43: 835-840.
Chang, S.L., 1947. Studies on Leptospira icterohaemorrhagiae. J.Inf.Dis., 81: 28.
Dalling, T. and Okell, C.C., 1926. Vaccine treatment of canine leptospiral jaundice. J.Path.Bact., 29: 131.
Desmecht, M., 1982. Durée de l'immunité conferée par le vaccin de la leptospirose canine chez le hamster. Ann.Méd.Vét., 126: 55-58.
Hanson, L.E., Tripathy, D.N. and Killinger, A.H., 1972. Current status of leptospirosis immunization in swine and cattle. J.A.V.M.A., 161: 1235-1243.
Hanson, L.E., 1973. Immunologic problems in bovine leptospirosis. J.A.V.M.A., 163: 919-921.
Hartman, E.G., 1977. Leptospirose. Tijdschr.Diergeneesk., 102: 45-52.
Hartman, E.G., van Houten, M., van der Donk, J.A. and Frik, J.F., 1984. Determination of specific anti-leptospiral immunoglobulins M and G in sera of experimentally infected dogs by solid-phase enzyme-linked immunosorbent assay. Vet.Immunol.Immunopathol., 7: 43-51.
Heath, K.R. and Box, P.G., 1965. Immunity to leptospirosis: Antibodies in vaccinated and infected dogs. J.Comp.Pathol., 75: 127-136.
Hood, L.E., Weissman, I.L. and Wood, W.B., 1978. Immunology. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California.
Huhn, R.C., Baldwin, C.D. and Cardella, M.A., 1975. Immunity to leptospirosis: Bacterins in dogs and hamsters. Am.J.Vet.Res., 36: 71-74.
Michna, S.W., 1970. Leptospirosis. Vet.Rec., 86: 484-496.
Negi, S.K., Myers, W.L. and Segre, D., 1971. Antibody response of cattle to Leptospira pomona: Response as measured by hemagglutination, microscopic agglutination, and hamster protection tests. Am.J.Vet.Res., 32: 1915-1920.
Ottosen, H.E., 1946. Om vaccination mod leptospirose hos hund. Medlemsbl. danske dylaegeforen., 29: 187-194.
Pike, R.M., 1967. Antibody heterogeneity and serological reactions. Bacteriol.Rev., 31: 157-174.
Pope, V., Hunter, E.F. and Feeley, J.C., 1982. Evaluation of the microenzyme-linked immunosorbent assay with Treponema pallidum antigen. J.Clin.Microbiol. 15: 630-634.
Tripathy, D.N., Smith, A.R. and Hanson, L.E., 1975. Immunoglobulins in cattle vaccinated with leptospiral bacterins. Am.J.Vet.Res., 36: 1735-1736.
Turner, L.H., 1968. Leptospirosis II. Trans.R.Soc.Trop.Med.Hyg., 62: 880-899.
World Health Organization, 1967. Current problems in leptospirosis research. Wld.Hlth.Org.techn.Rep.Ser., 380.