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SEROLOGICAL SURVEY OF CANINE PARVOVIRUS

LIOW SWEE LIAN

Serological Survey of Canine Parvovirus

By

LIOW SWEE LIAN

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To My Father and Mother

whose unselfish love, patience and understanding have taught their children the meaning of having a good education

To My Brothers and Sisters

for their love, encouragement and understanding

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ABSTRACT

Haemagglutination-inhibition (HI) test with 1% (V/V) porcine red blood cells was used to assay serum antibody (Ab) to canine parvovirus (CPV) in dogs which attended the clinics at Kuala Lumpur, Petaling Jaya, Kajang and Serdang. CPV Ab titres were analysed with respect to the age, sex, breed and vaccination status of dogs. Majority (90%) of the dogs vaccinated with modified live (ML) CPV had high serum HI Ab titres ($\geq$ 5120) whereas approximately 90% of non-vaccinated dogs and more than 90% of dogs of unknown vaccination history had serum HI titres $\geq$ 80 and $\geq$ 5120 respectively. Maternal serum HI Ab to CPV was present in non-vaccinated puppies up to 16 weeks of age. There was no significant difference in the levels of serum HI Ab to CPV in dogs of different ages, sexes and vaccination status. Majority ($\geq$ 95%) of the mongrels had protective level ($\geq$ 80) of serum HI Ab to CPV whereas approximately 10-15% of purebreed and crossbreed dogs had serum HI Ab to CPV below the protective level.

ABSTRAK

Ujian hemagglutinasi-inhibisi (HI) dengan menggunakan 1% (V/V) sel darah merah babi telah digunakan untuk mengasai antibodi (Ab) kepada parvo-virus anjing (CPV) di-dalam serum yang diambil daripada anjing-anjing yang mendapat rawatan di klinik sekitar Kuala Lumpur, Petaling Jaya, Kajang dan Serdang. Titer Ab CPV telah dianalisa bergantung pada umur, jantina, baka dan status vaksinasi anjing. Kebanyakan (90%) daripada anjing yang telah diberi vaksin dilemahkan CPV mempunyai titer Ab HI yang tinggi ($\geq$ 5120) di dalam serum. Manakala lebih kurang 90% daripada anjing yang tidak diberi vaksin atau yang tidak diketahui sejarah vaksinasi mempunyai titer HI $\geq$ 80 dan $\geq$ 5120 berasal dari ibu anjing terdapat didalam serum anak anjing yang tidak divaksinkan dengan CPV sehingga umurnya 16 minggu. Titer Ab HI kepada CPV di dalam serum-serum anjing yang berlainan umur, jantina dan status vaksinasi tidaklah mempunyai perbezaan yang penting. Kebanyakan ($\geq$ 95%) daripada anjing paria mempunyai titer-titer Ab HI yang sekurang-kurangnya ($\geq$ 80) dapat melindungi anjing daripada penyakit CPV, manakala lebihkurang 10-15% daripada anjing baka tulin dan anjing baka campuran mempunyai Ab HI kepada CPV yang tidak mencukupi untuk perlindungan daripada penyakit CPV.

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1. INTRODUCTION

Canine parvovirus (CPV) enteritis and myocarditis emerged as a new disease in dogs in 1978, with outbreaks of the disease reported in several countries (2,19,28,32,33,37). The disease, which is caused by a virus designated CPV type 2, is potentially fatal and highly contagious (10). Serological surveys conducted in many countries indicate that CPV infection occurs worldwide (1). In Malaysia, CPV infection was first reported in 1980 at the Universiti Pertanian Malaysia Small Animal Teaching Hospital, UPMSATH (20). Cases of suspected CPV enteritis seen at the UPMSATH were confirmed based on clinical and pathological findings (20,52) which suggested that CPV infection was present among the canine population. Since then, no study has been conducted to determine the prevalence of parvovirus enteritis and myocarditis the effectiveness of the vaccination programmes currently practised in the local canine population. The major objectives of this project are (a) to provide a preliminary study on the immune status of the canine population to CPV and (b) to determine the prevalence of CPV infection in the local canine population. This survey involved the collection of random serum samples from pet and stray dogs which inhabitated in or around Kuala Lumpur, Petaling Jaya, Kajang and Serdang areas. Serums were assayed for antibody (Ab) to CPV by using the haemagglutination-inhibition (HI) test and the Ab titres were analysed with respect to the age, sex, breed and vaccination status of dogs.

2. LITERATURE REVIEW

2.1 History of canine parvovirus (CPV) infection

CPV infection was first reported in the United States of America as a fatal, highly contagious viral disease of dogs in 1978 (2,19,39,49,53) Similar outbreaks occurred simultaneously in Australia (32,37,38). Canada (65), United Kingdom (33), New Zealand (28) and later in Malaysia (20,52).

Serological surveys indicated that the disease was present in the canine population in many parts of the world (1). Seroepidemiologic studies with serum collected from dogs prior to these outbreaks in 1977 and 1978 indicated that CPV was not present in the canine population before 1976 (59). Thus, CPV infection was established as a new viral disease in dogs (10,41).

CPV infection is caused by a virus designated CPV2 (8,10) which is a small (20nm) DNA virus of the family Parvoviridae (2,39). There are three recognised syndromes caused by CPV2 in dogs : (a) severe contagious enteritis that clinically and pathologically resembles feline panleucopaenia (FPV), (b) myocarditis leading to sudden death in puppies less than three months old (7,14,17,23,29,30,34,41,42,45,46,51,53) and (c) a generalised fatal neonatal disease (41,42,61). Serological and biochemical studies showed that CPV2 is closely related to FPV and mink enteritis virus (3,11, 39). However, the origin of CPV2 still remains speculative and may never be accurately determined (2,9,39,45,49).

2.2 Laboratory diagnosis of CPV infection

Haemagglutination (HA), serum virus neutralisation, virus isolation in tissue culture, immunofluorescence, electron microscopy (EM) and lately enzyme-linked immunosorbent assay can be used to detect CPV (3,11,22,31,35, 44,48,61,62). Laboratory dianosis of active CPV infection requires evidence of CPV shedding in faeces or evidence that the serum Ab to CPV is of recent origin, vaccination or previous infection with CPV could result in high circulating Ab titres which may persist for many months (49) HA test is the most widely used test for detecting CPV in faeces (44) and is twice as sensitive as virus isolation in tissue culture and 8 times as sensitive as EM (61). However, HA has two shortcomings; (a) the limited storage life of red blood cells (RBC) of porcine or rhesus macaque hence, requires frequent preparation of RBC suspensions and (b) the characteristics

ious donor pigs and rhesus macaque to show different sedi-

mentation qualities and cause variations in HA titres (11,40,44).

Porcine RBC is satisfactory for HA of CPV provided that proper selection of donor pigs is made and care is taken to avoid clotting (11). CPV agglutinates porcine and rhesus macaque RBC over a wide pH range (pH 5.8 - 7.4) at a low temperature (4°C); CPV will be eluted within a few hours if HA was allowed to stand at 22°C (11). There is an inverse relationship between porcine RBC concentrations and CPV HA titres; HA titres are reduced about twofold with each 0.5% increment in RBC concentration (11).

HI test, which is simple, rapid, sentitive and relatively inexpensive, has been used frequently for the detection of Ab to CPV (3,11). Conditions affecting HA also influence HI in that HI titres are inversely related to HA units (11). A shortcoming of HI test is the inability to distinquish between acute infection and past exposure when a single serum sample is tested (60). By using 2-mercaptoethanol to destroy immunoglobulin M, one can increase the specificity of HI test for detecting acute CPV infection (6,60).

2.3 Immunisation

2.3.1 Immunity

Investigators have found that there is a direct correlation between serum Ab titres and resistance to CPV infection (18,55,56). Circulating HI Ab to CPV at titres >80 are protective against CPV infection and prevent faecal shedding (9,50,55). Dogs with low HI titres (<40) become subclinically infected with virus shedding due to viral replication in the intestinal tract and gut-associated lymphoid tissue (39,56). Dogs that have recovered from CPV infection usually have HI titres (>640), are immune to reinfection for at least 20 months (9,49,56,57), and are conferred long-lived immunity (53).

2.3.2 Inactivated FPV and CPV vaccines

During the worldwide epidemic of CPV enteritis and myocarditis in 1978, the veterinary profession felt that there was a need for an effective immunisation programme to control the disease (43,46,54,67). Since by fortuity FPV is antigenically similar to CPV, inactivated and modified live FPV vaccines were used to immunise dogs against CPV (3,4,5,9,15,16,26,27,39,46,50,56,67). These feline origin vaccines were used, not as ideal immunising agents, but as expedients to protect a canine population that was largely susceptible due to limited prior exposure to CPV (4).

Both inactivated FPV and CPV vaccines provide protection against CPV infection for a finite period after immunisation (49). The titre of the virus in the vaccines as well as the interval between vaccinations influence the magnitude of the serologic response to inactivated vaccines (53). Maximal serologic HI response occurs within 1 to 2 weeks after vaccination (56). However, dogs with waning CPV Ab inactivated vaccines are less likely to develop clinical infection (16,56). Inactivated CPV vaccines can induce greater HI Ab responses than inactivated FPV vaccines (39,56,59). Inactivated CPV and FPV vaccines usually induce lower HI titres than attenuated FPV vaccines in vaccinated dogs (4,39,53,59). The antigenicity of the vaccine virus, the method of virus inactivation and the adjuvant used can influence the duration of immunity in vaccinated dogs given inactivated CPV and FPV vaccines (53,59). Increasing the titre of the virus in the inactivated CPV and FPV vaccines increases the initial Ab response but does not alter the rate of Ab decline (49).

2.3.3 Modified live (ML) FPV and CPV vaccines

The response of dogs to vaccination with ML FPV can be divided into two groups : (a) the development of modest CPV Ab titres following

vaccination which increase following a second vaccination and then decline in the absence of repeated vaccination and (b) the development of high CPV Ab titres which are not increased by a second vaccination and which persist in the absence of repeated vaccination, thus resemble dogs that have recovered from natural CPV infection (53). Dogs given FPV vaccine are immune against CPV (3,50,57). However, response of dogs to ML FPV is extremely variable because of the relatively limited ability of FPV to establish infection in the dog (27,53,57). It was found that the commercial ML FPV vaccines may not induce protective Ab titres in approximately one-third of all vaccinated dogs in the general canine population and could lead to vaccination failures (27).

ML CPV seems to provide the ultimate protection of dogs against CPV infection (4,12,13,26,36). ML CPV has been shown to induce a more uniform immune response using a small immunising dose (49). CPV HI titre engendered by ML CPV is superior to that induced by inactivated CPV, inactivated FPV and ML FPV (49). Dogs given ML CPV can develop HI titres as early as 2 days postvaccination (13). Maximal CPV Ab titres, which can occur within one week postvaccination, are comparable to those observed after natural CPV infection (13,36). Immunisation with ML CPV consistently results in HI Ab titres >320 that persist for at least 2 years (14,12,13,36,53). ML CPV has the ability to prevent clinical illness and subclinical infection as well as virus shedding in faeces (4,13). The serologic response of dogs given ML CPV increase in dogs with HI titres <10 but decreases reciprocally in those with HI titres >80 at the time of vaccination (13).

2.4 Role of maternal Ab to CPV

Maternal Ab has an important role in protection of neonates against disease. CPV Ab from a recovered or successfully vaccinated bitch is transferred to her puppies via the placenta and colostrum (49). However,

colostral transfer accounts for approximately 90% of the maternal CPV Ab (47,53,55). Thus transplacental transfer of CPV Ab is of secondary importance to colostral transfer and colostrum-derived puppies would be refractory to immunisation or infection for several weeks (55). The amount of CPV Ab that puppies receive via placenta and colostrum is directly proportional to the Ab titre of the bitch and is equivalent to 50% of the bitch's serum Ab titre after suckling (55). Within 3 days postpartum, HI titres in puppies are usually equal to that of the bitch (53). Maternal CPV Ab level in puppies declines exponentially with time; the half-life is about 9 days (53,55). The passively derived CPV Ab is protective against CPV infection when HI titres >80 (9,50,55,56); HI Ab titres <40 do not provide immunity to CPV infection (56). Maternal CPV Ab is the most important cause of CPV immunisation failures among puppies (13,26,56,59). Serologic response to vaccination with ML FPV, inactivated FPV and CPV is suppressed in puppies that have demonstrable maternal CPV Ab at the time of vaccination (55). Suppression of humoral response to ML CPV may occur in puppies up to 16 weeks of age when maternal CPV Ab >20 (13,55).

3. MATERIALS AND METHOD

3.1 Cells and Media

A feline embryo (FEmb) cell line (a gift from Dr. M.J. Studdert, Melbourne, Australia) was used. The complete growth medium (FEL) was Eagle's basal medium (Flow Laboratories, North Ryde, N.S.W., Australia) modified to contain twice the usual amounts of amino acids and vitamins, 10% tryptose phosphate broth and 10% fetal calf serum (Flow Laboratories, North Ryde, N.S.W., Australia). Confluent monolayers of FEmb cells, passage 50 to 52, were placed on serum-free FEL medium for 24 hours of synchronisation. Synchronised FEmb cells were trypsinised, suspended in 80 ml of complete growth medium at 2×10^5 cells/ml in Roux flask and incubated at 37°C 18 hours prior to virus inoculation

3.2 Virus

The CPV strain (V37/84) used was isolated from the intestinal content of a one-year old German Shepherd with haemorrhagic enteritis. This strain was identified as a parvovirus (63). A stock of CPV strain V37/84 of passage VII was prepared in synchronised FEmb cells in 3 Roux flasks with complete growth medium as previously described (40,Tham, personal communication). CPV-infected FEmb cells were further incubated at 37°C for 2 to 3 days and were frozen at -70°C when complete cytopathic effect was achieved. The cells were frozen and thawed 3 times, pooled and clarified by low speed (1500 rpm/15 minutes) centrifugation. The supernates were transferred into sterile bottles, with an aliquot removed and assayed for HA activity. Clarified CPV stock solution was stored at -70°C until required.

3.3 Serums

Serums were collected from dogs which came for routine check-up or treatment at the UPMSATH in Serdang and Petaling Jaya, at a government veterinary clinic in Kajang and at a private veterinary clinic in Kuala Lumpur. Serums were also taken from dogs housed at the Society for the Prevention of Cruelty to Animals in Kuala Lumpur. All serums were collected at these clinics from 9th July, 1985 to 26th November, 1985.

Dogs more than 6 weeks old were bled from the cephalic vein. For those less than 6 weeks of age, jugular vein was used. Blood was collected into 5-ml plain venoject tubes using sterile 1½ inch 21-gauge hypodermic needles and 5-ml syringes. Following clotting, blood samples were centrifuged at 1500 rpm for 10 minutes at 4°C. Serums were then collected into sterile 2-ml bottles. Sterility was observed during transfer of serums from the tubes to the bottles. Serums were heat-inactivated in a water-bath at 56°C for 30 minutes and stored at -20°C until HI test was performed All serums were diluted 1:10 with phosphate buffer saline (PBS) pH 6.8 for HI test.

3.4 Preparation of 1% (V/V) porcine red blood cells (RBC) suspension

Weaning piglets of Landrace cross (Puchong Farm, Universiti Pertanian Malaysia) were bled from the right jugular vein using 1½ inch 21-gauge hypodermic needles and 10-ml syringes containing approximately 1 ml of sterile Alsever's solution (Appendix I). Blood was collected into an equal volume of ice-cold Alsever's solution with care to avoid clotting. Porcine RBC were then washed with cold phosphate-buffered saline (PBS) solution of pH 6.8 (Appendix II) by centrifugation at 1500 rpm for 10 minutes. The supernatent was poured off and porcine RBC were resuspended in PBS pH 6.8. The process of washing was repeated 3 times. A 10% (V/V) suspension of porcine RBC in ice-cold PBS pH 6.8 was prepared and stored at 4°C for not more than 7 days. One percent (V/V) porcine RBC suspension in ice-cold PBS pH 6.8 containing 1% fetal bovine serum was prepared fresh when required Routinely 3 or 4 weaning piglets of Landrace cross were bled and their bloods were screened to determine its suitability for HA. Blood from the predetermined suitable donor i.e. pig whose blood was haemagglutinated at the highes dilution of CPV strain V37/84, was used in the subsequent HI test.

3.5 Haemagglutination (HA) titration

The procedures of HA described by Lenghaus (40) were followed with slight modification. Briefly, 0.05 ml of PBS pH 6.8 was added, in duplicate from well 2 to well 12 in a 96-well V-bottom microtitre plate (Dynatech Laboratories, Alexandria, Virginia, U.S.A.). 0.1 ml of CPV strain V37/84 VII (see 3.2) was added to the first well in duplicate. A serial 2-fold dilution of the virus was carried out from well 1 to well 11 using 0.05 ml diluting loops, leaving the last well as a cell control. 0.05 ml of ice-cold 1% (V/V) porcine RBC was added to all the wells. The plate was covered with a loose-fitting lid and incubated at 4°C for about an hour. HA was read after one hour of incubation and the endpoint was expressed as the highest dilution of the virus with complete haemagglutination. HA test was

performed with all the materials maintained at 4°C at all time.

3.6 Haemagglutination-inhibition (HI) test

The procedures of HI described by Lenghaus (40) was followed. Briefly, 0.025 ml of PBS pH 6.8 was added in all the wells in a 96-well V-bottom microtitre plate. Wells 1 and 12 were added 0.025 ml of diluted heat-inactivated serums. Serial 2-fold dilution of serum was made from well 1 and well 11 with 0.025 ml dilution loops. 0.025 ml of CPV strain V37/84 VII diluted in PBS pH 6.8 to contain 4 HA units was added to all wells except well 12. The plate was then incubated at room temperature for one hour and chilled at 4°C for 5 minutes. 0.05 ml of ice-cold 1% porcine RBC was then added to each well and the plate was further incubated at 4°C for an hour. HI was read 1 hour incubation at 4°C and the results were expressed as the reciprocal of the highest dilution of serum that completely inhibited 4 HA units of the virus. Controls included HA titration of the test virus, wells containing serum alone i.e. without virus and the pig RBC suspension.

4. RESULTS

A total of 151 serum samples were collected and assayed for CPV Ab.

Dogs were categorised as vaccinated, non-vaccinated and unknown vaccination history. Dogs with unknown vaccination history were mostly strays. Majority of dogs in all the 3 groups had HI titres > 5120 (Fig.1). The vaccinated group comprised of 50 dogs (33%) which had HI titres > 80; approximately 90% of the vaccinated dogs had HI titres > 5120 (Fig.1). Serologic response (HI titres) of dogs with respect to the age and time after CPV vaccination is shown in Table 1. Fifteen vaccinated dogs had no date of vaccination but had HI titres > 80. The serum HI Ab response of dogs given 2 commercial ML CPV vaccines (A and B) is shown in Table 2.

The non-vaccinated group comprised of 65 dogs (43%) of which about 90% had HI titres > 80 (Fig.1). Table 3 shows CPV HI titres in non-vaccinated dogs of different ages. Eleven cases of diarrhoea which were either watery, blood-tinged or haemorrhagic were recorded; most of these cases were attributed to

gastrointestinal parasitism. In another 3 diarrhoeic cases, CPV infection was suspected; one was positive for CPV enteritis in which a 3-month old dog with a prevaccination HI titre of 320 died 6 days after vaccination with ML CPV (Norden Laboratories Lincoln, USA).

Thirty-six dogs (24%) with unknown vaccination history had HI titres ranged from 40 to 204, 800 (Fig.1). Ninety-four per cent of these dogs had HI titres > 5120; the remainder had HI titres < 80.

Z-test at 5% confidence level showed that there was no significant difference in serum HI titres between vaccinated and non-vaccinated dogs. To avoid bias, serum HI titres of dogs with unknown vaccination history were not compared in the Z-test.

Dogs from which serums were collected were divided into 6 age groups. Fig. 2A and 2B show serum HI titres of dogs in each age group; more than 90% of the dogs in the 6 age-groups had HI titres > 80. Of particular interest were dogs less than 6 weeks and between 6 weeks to 3 months of age; majority (>70%) of these dogs had HI titres >5120. Analysis of Variance at 5% confidence level showed that there was no significant difference in serum HI titres among the age groups.

Breeds of dogs in this survey were categorised into crossbreds, purebreds and mongrels. Purebreds include German Shepherd, Spitz, Dobermann, Bull Mastiff Dalmatian, Bull Terrier and Labrador. Crossbreds include Spitz X, Dobermann X, Bull Mastiff X and Bull Terrier X. The distribution of these breeds with respect to serum HI titres is shown in Fig. 3. The majority (80%) of all breeds had HI titres > 80. Analysis of Variance at 5% confidence level and Duncan New Multiple Range Test showed that mongrels had significantly higher serum HI titres to CPV than purebreds; no significant difference in serum HI titres was found between mongrels and crossbreds and also between purebreds and crossbreds

Serum HI titres of male and female dogs are shown in Fig. 4. More than 90% of dogs of both sexes had HI titres ≥ 80; 83% of male and 71.5% of female dogs

had HI titres > 5120. Z-test at 5% confidence level showed no significant difference in serum HI titres of both male and female dogs.

5. DISCUSSION

Although the number of dogs in this serological survey was small, the findings are significant for the canine population in the areas under study.

Majority (>90%) of the dogs surveyed had HI titres ≥ 80 which adequately protect the animals against CPV infection. It appears that natural CPV infection is present in the local canine population as suggested by high CPV serum HI Ab titres in stray and pet dogs which had not been vaccinated against CPV.

Most dogs which had been vaccinated with ML CPV developed high CPV serum HI Ab levels which persisted for long period of time following vaccination (Figure 1 and Table 1). This reflects the ability of ML CPV in activating a good immune response (Table 2). However, the effectiveness of the various commercial ML CPV vaccines used in the local canine population to induce protective humoral immunity is difficult to assess herein, because prevaccination HI titres were not available and the number of serum samples collected was small It must be stressed that further studies with large number of dogs, preferably with known prevaccination Ab titres, are necessary to make a fair comparison on the effectiveness of the several CPV vaccines available locally. This is particularly important since a 3-month old puppy with a prevaccination serum HI titre of 320 died of CPV enteritis 6 days after ML CPV vaccination. This is probably due to a vaccination breakdown, as a result of interference from the prevaccination CPV serum HI Ab (presumably maternal Ab) together with concurrent exposure to CPV or incubation of CPV infection at time of vaccination (64). It is known that 4 to 8 weeks old puppies with high maternal CPV Ab levels may develop a suppressed humoral response to ML CPV and may lead to vaccination breakdowns when maternal CPV Ab > 20 (13).

This survey shows that majority of puppies below 16 weeks of age which had not been vaccinated against CPV had high serum HI Ab titres (Table 3); these Abs

are probably of maternal origin. This is in agreement with the findings of Pollock and Carmichael (55) in which puppies born to bitches recovered from CPV infection had high maternal CPV Ab which suppressed humoral response to CPV vaccination until the puppies were 14 to 16 weeks of age. As it is common to vaccinate puppies against CPV at 12 weeks old, a proportion of the puppies would be left unprotected. Age is a determining factor in the development of immunity against CPV infection (59). Puppies are fully capable of mounting immune responses at birth, but they require maternal CPV Ab for protection against CPV infection until they can mount a protective humoral immunity (59,66).

McCandlish (45) suggested that all breeds of dogs were susceptible to CPV infection. In contrast, the study of susceptibility of breeds of dogs to CPV by Rowley (58), which was based on the number of positive cases of CPV infection according to breeds, was inconclusive. On the basis of HI titres (Figure 3), the findings of this survey showed that majority (≥95%) of mongrels had protect serum HI titres (>160) and were presumably less susceptible to CPV infection. Approximately 10-15% of purebreed and crossbreed dogs surveyed herein had serum HI titres below the protective level of 80 and were thus perceptibly more susceptible to CPV infection. Mongrels make up the majority of the pet and stray dog populations in Malaysia (21). Mongrel stray and pet dogs which are usually unleashed and tend to run in packs (21) are thus exposed to CPV infection in contaminated environment; these dogs either die or recover from the disease. Hence mongrels that recover from CPV infection would have high serum HI titres as reported (13,56,57,61). In contrast, the small proportion of non-vaccinated purebreed and crossbreed dogs which had serum HI titres < 40 (Table 4) were subclinically infected with CPV (39,55), probably via fomites (49).

6. CONCLUSION

1. CPV infection is widespread in the local canine population and can be fatal in young dogs.

2. Majority of the dogs surveyed herein have high CPV serum HI Ab levels as a result of vaccination, natural infection or passive immunity.
3. ML CPV vaccines appear to be effective in the induction of serum HI Ab.
4. Maternal CPV serum HI Ab is present in puppies up to 16 weeks of age.
5. Serum HI Ab to CPV are present in the majority of dogs at comparable levels irrespective of age, sex and vaccination.
6. Majority of the mongrels (≥95%) have protective level (≥80) of serum HI Ab to CPV whereas approximately 10-15% of purebreed and crossbreed dogs have serum HI Ab to CPV below the protective level.

FIGURE 1

Canine Parvovirus Serum Haemagglutination - inhibition (HI)
Antibody Titres of 151 Dogs of Different Vaccination History

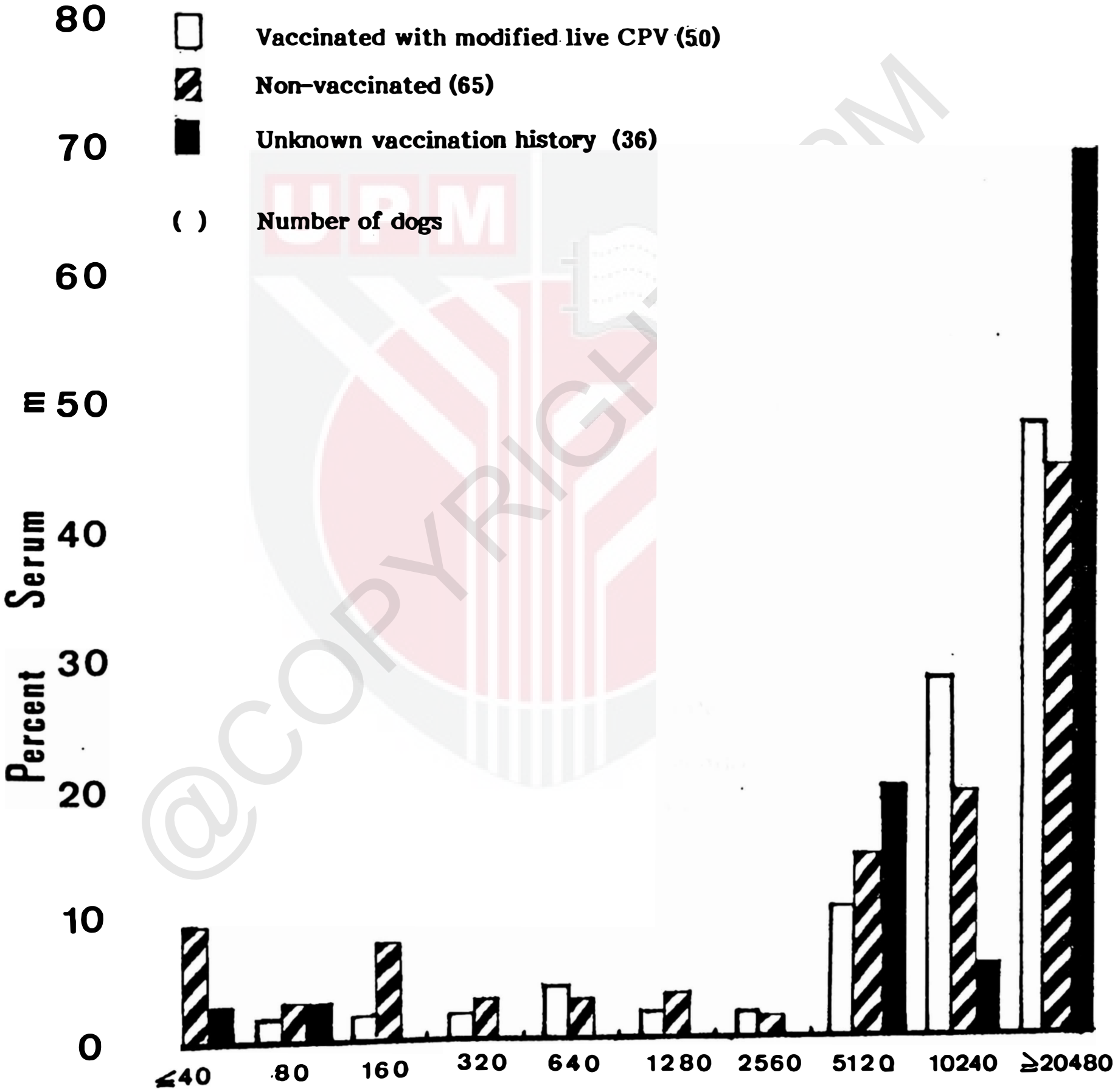
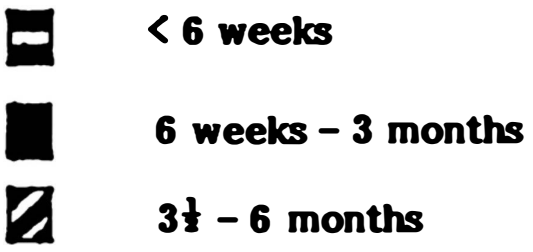


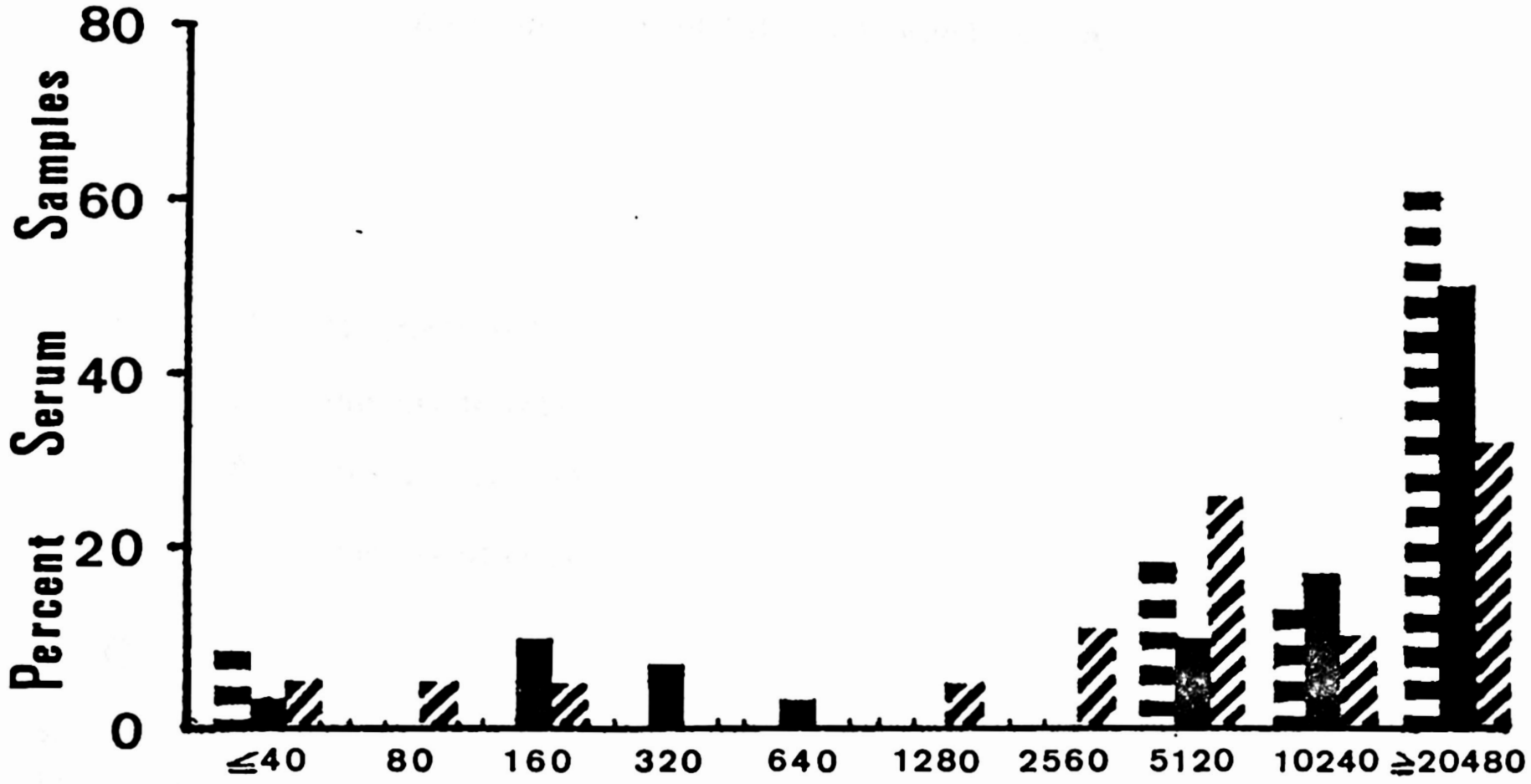
FIGURE 2

Canine Parvovirus Serum Haemagglutination – inhibition (HI)

Antibody Titres of Dogs of Different Ages



A


Percent Serum Samples
80
60
40
20
0
≤40
80
160
320
640
1280
2560
5120
10240
≥20480
HI Titre

B

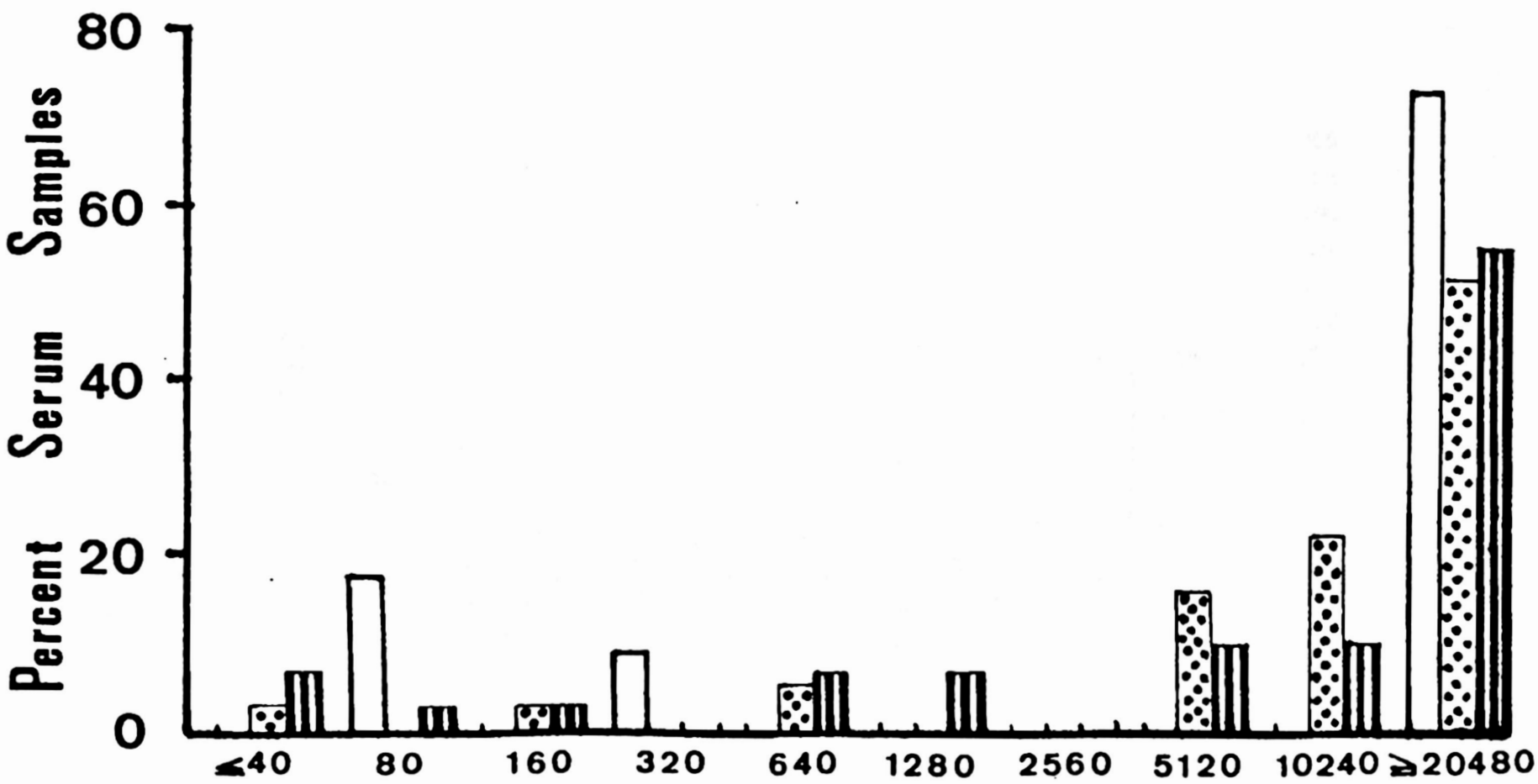

Percent Serum Samples
80
60
40
20
0
≤40
80
160
320
640
1280
2560
5120
10240
≥20480
HI Titre

FIGURE 3

Canine Parvovirus Serum Haemagglutination – inhibition (HI)

Antibody Titres of Different Breeds of Dogs

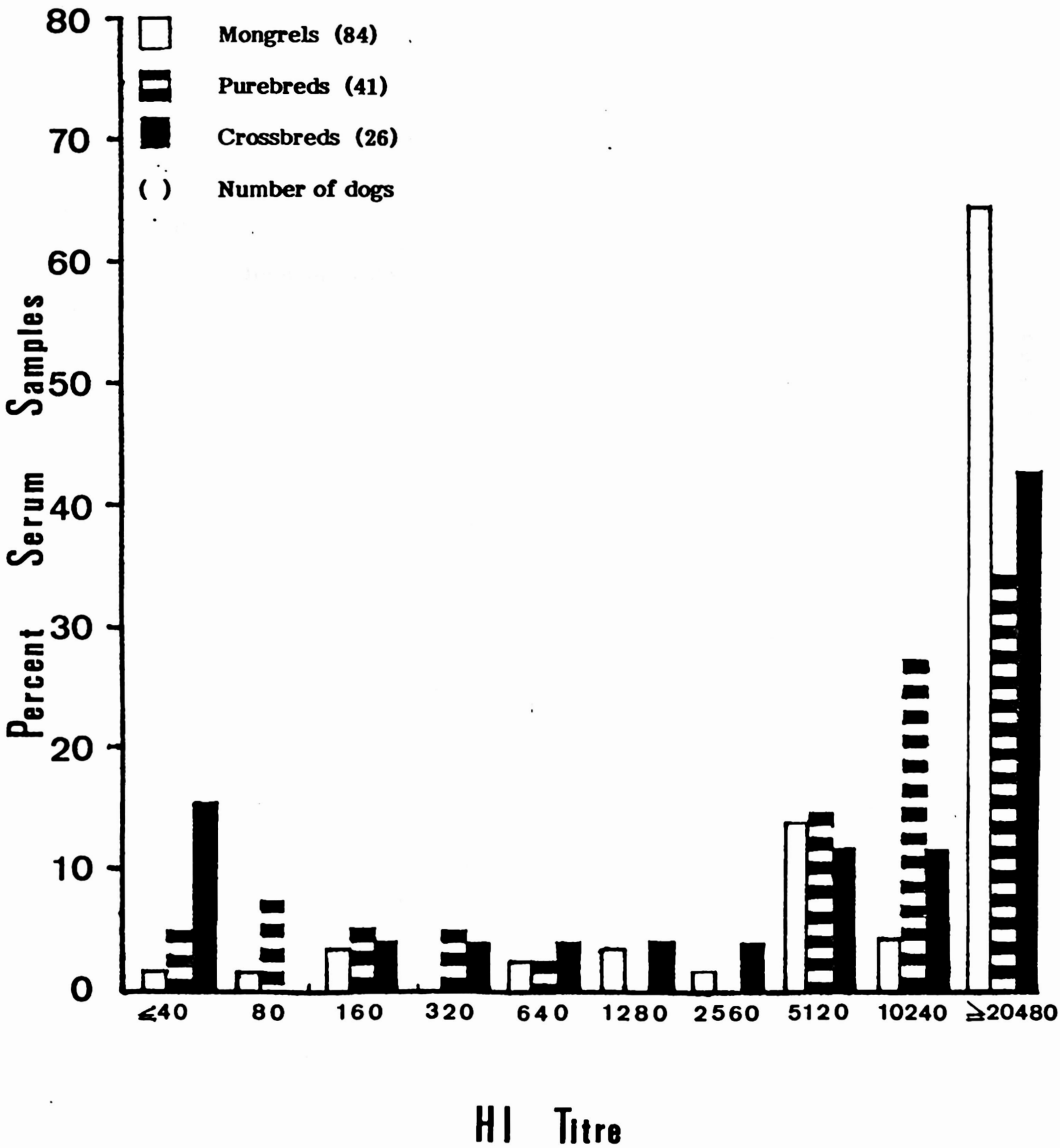


FIGURE 4

Canine Parvovirus Serum Haemagglutination – inhibition (HI)

Antibody Titres of Male and Female Dogs (151)

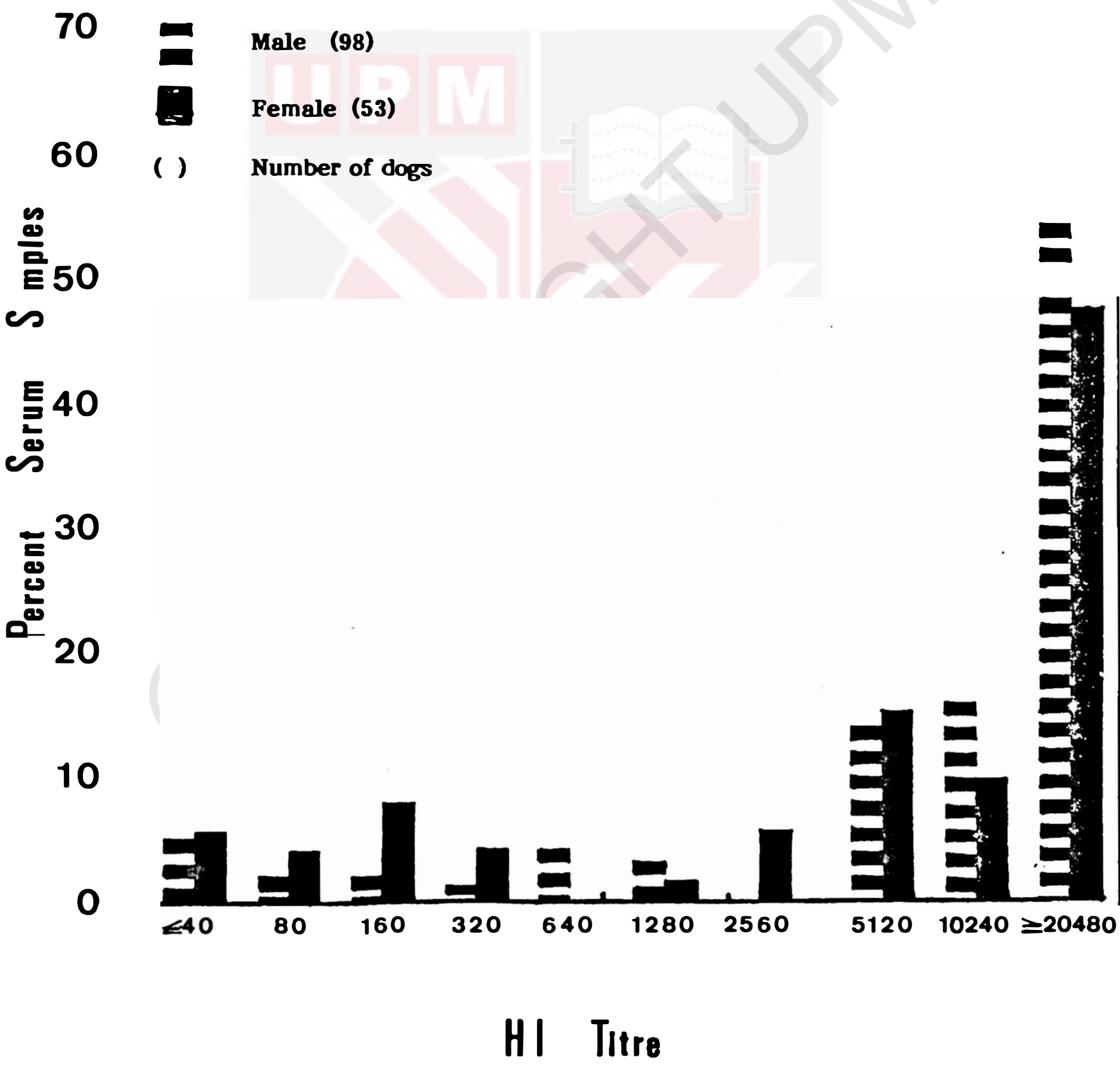


TABLE 1: Serum Haemagglutination Inhibition Antibody Titres of Dogs of Various Ages Following Vaccination With Modified Live Canine Parvovirus

Age at Vaccination	Time After Vaccination	HI Titre	Number of Serum Samples
8 wk*	1 d	3,200 - 204,800	5
4 - 6 wk	4 - 6 d	3,200 - 204,800	9
12 - 14 wk	20 - 28 d	1,280 - 51,200	3
2 yr*	4 - 30 d	6,400 - 10,240	2
4 mo - 3 yr	2 - 4 mo*	2,560 - 204,800	7
6 mo - 4 yr	1 yr	640 - 25,600	5
2 - 3 yr	2 yr	3,200 - 51,200	4

* d = day; wk = week; mo = month; yr = year

TABLE 2: Serum Haemagglutination Inhibition Antibody Titres of Dogs Immunised With Modified Live Canine Parvovirus Vaccines A and B

Vaccine	Number of Serum Samples	Age (month)	Time Postvaccination (month)	HI Titre
A	3	3 - 3½	1	1280; 12,800; 51,200
B	2	4	2	5120; 6400

TABLE 3: Canine Parvovirus Serum Haemagglutination Inhibition Titres of Non-Vaccinated Dogs of Different Ages

Age Group*	HI Titre									
	0	40	80	160	320	640	1280	2560	5120	10240
0 - 5 wk (13)	15**	--	--	--	--	--	--	--	23	62
6 - 16wk (22)	4.5	4.5	4.5	18.2	9	4.5	--	--	13.6	41.2
17wk - 6 mo (3)	--	--	--	--	--	--	--	33.3	33.3	33.4
6½ - 12mo (3)	--	33.3	--	--	--	--	--	--	--	66.7
12 - 24mo (3)	--	--	--	--	--	--	--	--	33.3	66.7
24mo (17)	--	11.8	6	6	--	11.8	11.8	--	6	46.6

* wk = week; mo = month

** Values expressed in percent

( ) Total number of serum samples per age group

TABLE 4: Canine Parvovirus Serum Haemagglutination Inhibition Titres of Non-Vaccinated Dogs of Different Breeds

Breed	Number of Serum Samples	HI Titre
Mongrel	34	0 - 204,800
Purebred	14	10 - 102,400
Crossbred	16	0 - 204,800

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APPENDIX I

ALSEVER'S SOLUTION

Glucose	2.05 g
Sodium chloride (NaCl)	0.42 g
Trisodium citrate ($Na_3C_6H_5O_7.2H_2O$)	0.80 g
Citric acid ($C_3H_4(COOH)_3.H_2O$)	0.55 g

Make up to 100 ml in distilled water, dispense into 20-ml bottles, autoclave at 15 lb. for 15 minutes and store at 4°C.

APPENDIX II

PHOSPHATE-BUFFERED-SALINE (PBS) - Calcium and Magnesium free

Sodium chloride (NaCl)	8.0 g
Potassium chloride (KCl)	0.2 g
Disodium-o-hydrogen phosphate (Na_2HPO_4)	1.15 g
Potassium-o-dihydrogen phosphate (KH_2PO_4)	0.2 g

Make up to 1000 ml with distilled water, sterilize by autoclaving at 15 lb for 15 minutes. Final pH 7.2 - 7.4. Adjust to pH 6.8.

APPENDIX III

NUMBER OF PUREBRED AND CROSSBRED DOGS INVOLVED IN THE CANINE PARVOVIRUS SEROLOGICAL SURVEY

PUREBREDS	NUMBER OF DOGS
German Shepherd	13
Spitz	11
Dobermann	8
Bull Mastiff	1
Bull Terrier	1
Dalmatian	1
Labrador	6
	41

CROSSBREDS	NUMBER OF DOGS
German Shepherd X	8
Spitz X	10
Dobermann X	4
Bull Mastiff X	1
Bull Terrier X	2
Labrador X	1
	26

APPENDIX IV

CANINE PARVOVIRUS SERUM HAEMAGGLUTINATION-INHIBITION (HI) ANTIBODY TITRES OF 151 DOGS OF DIFFERENT VACCIANATION HISTORY

A. Vaccinated With Modified Live Canine Parvovirus

Total number of serum samples = 50 (33%)

Serum Sample No.	HI Titre
UPM 3/85	6,400
UPM 4/85	10,240
UPM 9/85	640
UPM 16/85	3,200
UPM 30/85	12,800
UPM 33/85	160
UPM 35/85	80
UPM 36/85	400
UPM 37/85	320
UPM 38/85	10,240
UPM 39/85	10,240
UPM 52/85	10,240
UPM 53/85	10,240
UPM 56/85	25,600
UPM 63/85	10,240
UPM 64/85	25,600
PJ 1/85	25,600
PJ 2/85	25,600
PJ 4/85	3,200
PJ 6/85	6,400
PJ 7/85	10,240
PJ 12/85	2,560
KA 4/85	6,400
KA 5/85	5,120
KA 13/85	12,800
KA 14/85	6,400
KA 15/85	51,200
KA 8/85	25,600
MDH 2/85	1,280
MDH 3/85	204,800
MDH 4/85	204,800
MDH 5/85	10,240
MDH 6/85	51,200
MDH 7/85	12,800
MDH 9/85	204,800
SP 2/85	102,400
SP 4/85	204,800
SP 6/85	204,800
SP 10/85	102,400
SP 11/85	204,800
SP 20/85	12,800
SP 21/85	3,200
SP 22/85	25,600
SP 26/85	51,200
SP 28/85	12,800
SP 30/85	25,600
SP 31/85	3,200
SP 32/85	6,400
SP 34/85	6,400
SP 38/85	102,400

C. Unknown vaccination history

Total number of serum samples = 36 (24%)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 17/85	3,200	SP 1/85	51,200
UPM 18/85	12,800	SP 3/85	102,400
UPM 19/85	6,400	SP 5/85	204,800
UPM 20/85	3,200	SP 7/85	204,800
UPM 21/85	5,120	SP 8/85	204,800
UPM 22/85	12,800	SP 9/85	102,400
UPM 23/85	12,800	SP 13/85	3,200
UPM 28/85	40	SP 18/85	25,600
UPM 29/85	80	SP 19/85	102,400
UPM 41/85	20,480	SP 23/85	25,600
UPM 44/85	12,800	SP 24/85	25,600
UPM 45/85	12,800	SP 25/85	25,600
UPM 47/85	25,600	SP 27/85	102,400
UPM 59/85	12,800	SP 29/85	25,600
UPM 61/85	3,200	SP 37/85	25,600
		SP 41/85	3,200
PJ 10/85	5,120	SP 42/85	51,200
PJ 13/85	25,600		
PJ 17/85	102,400		
KA 1/85	10,240		

APPENDIX V

CANINE PARVOVIRUS HAEMAGGLUTINATION-INHIBITION (HI) ANTIBODY TITRES OF DOGS OF DIFFERENT AGES

A. Less than 6 weeks old

Total number of serum samples = 23 (15.2%)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 5/85	0	SP 28/85	12,800
UPM 8/85	0	SP 30/85	25,600
		SP 32/85	6,400
SP 2/85	102,400	SP 33/85	51,200.
SP 4/85	204,800	SP 35/85	25,600
SP 11/85	204,800	SP 36/85	3,200
SP 12/85	102,400	SP 38/85	102,400
SP 14/85	25,600	SP 39/85	25,600
SP 15/85	3,200	SP 40/85	6,400
SP 16/85	12,800	SP 43/85	25,600
SP 17/85	3,200	SP 44/85	6,400
SP 21/85	3,200		
SP 26/85	51,200		

B. Between 6 weeks and 3 months

Total number of serum samples = 30 (19.9%)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 14/85	160	UPM 43/85	520
UPM 15/85	12,800	UPM 55/85	25,600
UPM 16/85	3,200	UPM 63/85	10,240
UPM 24/85	160	UPM 65/85	3,200
UPM 25/85	320		
UPM 26/85	320	PJ 3/85	204,800
UPM 31/85	40,960		
UPM 34/85	40	MDH 1/85	160
UPM 41/85	20,480	MDH 6/85	51,200
UPM 42/85	6,400	MDH 7/85	12,800

Serum Sample No.	HI Titre
MDH 8/85	102,400
KA 1/85	102,400
KA 7/85	10,240
KA 11/85	102,400
SP 1/85	51,200
SP 6/85	204,800
SP 10/85	102,400
SP 20/85	12,800
SP 22/85	25,600
SP 23/85	25,600
SP 31/85	3,200
SP 34/85	6,400

C. Between 3½ months and 6 months

Total number of serum samples = 19 (12.6%)

Serum Sample No.	HI Titre
UPM 6/85	80
UPM 7/85	2,560
UPM 12/85	160
UPM 32/85	0
UPM 49/85	5,120
UPM 50/85	51,200
UPM 51/85	20,480
UPM 52/85	10,240
UPM 58/85	5,120
UPM 64/85	25,600
UPM 66/85	25,600
PJ 5/85	5,120
PJ 12/85	2,560
PJ 14/85	3,200
MDH 2/85	1,280
MDH 11/85	204,800
KA 4/85	6,400
KA 5/85	5,120
KA 8/85	25,600

D. Between 6½ months and 12 months

Total number of serum samples = 11 (7.3%)

Serum Sample No.	HI Titre
UPM 29/85	80
UPM 35/85	80
UPM 37/85	320
UPM 56/85	25,600
PJ 2/85	25,600
KA 12/85	25,600
KA 13/85	12,800

Serum Sample No.	HI Titre
MDH 4/85	204,800
MDH 9/85	204,800
MDH 10/85	204,800
SP 24/85	25,600

E. Between 12½ months and 24 months

Total number of serum samples = 37 (24.5%)

Serum Sample No.	HI Titre
UPM 1/85	10
UPM 3/85	6,400
UPM 4/85	10,240
UPM 17/85	3,200
UPM 18/85	12,800
UPM 19/85	6,400
UPM 20/85	3,200
UPM 21/85	5,120
UPM 22/85	12,800
UPM 23/85	12,800
UPM 33/85	160
UPM 36/85	400
UPM 38/85	10,240
UPM 39/85	10,240
UPM 44/85	12,800
UPM 53/85	10,240
UPM 54/85	10,240
UPM 59/85	12,800
UPM 60/85	20,480
UPM 62/85	51,200
UPM 9/85	640
PJ 10/85	5,120
PJ 13/85	25,600
MDH 3/85	204,800
KA 3/85	10,240
KA 15/85	51,200
SP 7/85	204,800
SP 8/85	204,800
SP 9/85	102,400
SP 13/85	3,200
SP 19/85	102,400
SP 25/85	25,600
SP 27/85	102,400
SP 29/85	25,600
SP 37/85	25,600
SP 41/85	3,200
SP 42/85	51,200

F. More than 24 months

Total number of serum samples = 31 (20.5%)

Serum Sample No.	HI Titre
UPM 2/85	40
UPM 10/85	160
UPM 11/85	1,280
UPM 13/85	640
UPM 27/85	80
UPM 28/85	40
UPM 30/85	12,800
UPM 40/85	800
UPM 45/85	12,800
UPM 46/85	12,800
UPM 47/85	25,600
UPM 48/85	102,400
UPM 57/85	51,200
UPM 61/85	3,200
UPM 67/85	25,600

Serum Sample No.	HI Titre
SP 3/85	102,400
SP 5/85	204,800
SP 18/85	25,600
PJ 1/85	25,600
PJ 4/85	3,200
PJ 6/85	6,400
PJ 7/85	102,400
PJ 11/85	1,280
PJ 15/85	5,120
PJ 16/85	12,800
PJ 17/85	102,400
MDH 5/85	10,240
KA 6/85	12,800
KA 9/85	102,400
KA 10/85	51,200
KA 14/85	6,400

APPENDIX VI

CANINE PARVOVIRUS SERUM HAEMAGGLUTINATION-INHIBITION (HI) ANTIBODY TITRES OF DIFFERENT BREEDS OF DOGS

A. Mongrels

Total number of serum samples = 84 (55.7%)

Serum Sample No.	HI Titre
UPM 8/85	0
UPM 10/85	160
UPM 11/85	1,280
UPM 12/85	160
UPM 13/85	640
UPM 15/85	12,800
UPM 17/85	3,200
UPM 18/85	12,800
UPM 19/85	6,400
UPM 20/85	3,200
UPM 21/85	5,120
UPM 22/85	12,800
UPM 23/85	12,800
UPM 29/85	80
UPM 30/85	12,800
UPM 33/85	160
UPM 40/85	800
UPM 42/85	6,400
UPM 43/85	520
UPM 46/85	12,800
UPM 47/85	25,600
UPM 48/85	102,400
UPM 51/85	20,480
UPM 55/85	25,600
UPM 57/85	51,200
UPM 58/85	5,120
UPM 62/85	51,200
UPM 66/85	25,600

Serum Sample No.	HI Titre
MDH 3/85	204,800
MDH 4/85	204,800
MDH 9/85	204,800
KA 1/85	10,240
KA 10/85	51,200
KA 11/85	102,400
PJ 2/85	25,600
PJ 3/85	204,800
PJ 11/85	1,280
PJ 12/85	2,560
PJ 15/85	5,120
PJ 16/85	12,800
SP 1/85	51,200
SP 2/85	102,400
SP 3/85	102,400
SP 4/85	204,800
SP 5/85	204,800
SP 6/85	204,800
SP 7/85	204,800
SP 8/85	204,800
SP 9/85	102,400
SP 10/85	102,400
SP 11/85	204,800
SP 12/85	102,400
SP 13/85	3,200

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
SP 14/85	25,600	SP 30/85	25,600
SP 15/85	3,200	SP 31/85	3,200
SP 16/85	12,800	SP 32/85	6,400
SP 17/85	3,200	SP 33/85	51,200
SP 18/85	25,600	SP 34/85	6,400
SP 19/85	102,400	SP 35/85	25,600
SP 20/85	12,800	SP 36/85	3,200
SP 21/85	3,200	SP 37/85	25,600
SP 22/85	25,600	SP 38/85	102,400
SP 23/85	25,600	SP 39/85	25,600
SP 24/85	25,600	SP 40/85	6,400
SP 25/85	25,600	SP 41/85	3,200
SP 26/85	51,200	SP 42/85	51,200
SP 27/85	102,400	SP 43/85	25,600
SP 28/85	12,800	SP 44/85	6,400
SP 29/85	25,600		

B. Purebreds

Total number of serum samples = 41 (27.1%)

i. German Shepherd (13)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 4/85	10,240	KA 4/85	6,400
UPM 27/85	80	KA 5/85	5,120
UPM 28/85	40	KA 9/85	102,400
UPM 56/85	25,600	KA 15/85	51,200
UPM 59/85	12,800		
UPM 63/85	10,240	PJ 7/85	102,400
UPM 67/85	25,600	PJ 13/85	25,600

i. Spitz (11)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 1/85	10	MDH 1/85	160
UPM 6/85	80	MDH 8/85	102,400
UPM 14/85	160		
UPM 54/85	10,240	PJ 1/85	25,600
UPM 65/85	3,200	PJ 6/85	6,400
		PJ 10/85	5,120
KA 3/85	10,240		

ii. Dobermann (8)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 16/85	3,200	KA 6/85	12,800
UPM 52/85	10,240	KA 8/85	25,600
UPM 61/85	3,200	KA 14/85	6,400
MDH 6/85	51,200		
MDH 7/85	12,800		

v. Labrador (6)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 3/85	6,400	UPM 37/85	320
UPM 35/85	80	UPM 38/85	10,240
UPM 36/85	400	UPM 39/85	10,240

. Bull Mastiff (1)

Serum Sample No.	HI Titre
UPM 25/85	320

vi. Dalmatian (1)

Serum Sample No.	HI Titre
PJ 14/85	3,200

ii. Bull Terrier (1)

Serum Sample No.	HI Titre
PJ 17/85	102,400

Crossbreds

Total number of serum samples = 26 (17.2%)

German Shepherd X (8)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 2/85	40	MDH 11/85	204,800
UPM 24/85	160		
UPM 41/85	20,480	KA 7/85	10,240
		KA 12/85	25,600
PJ 5/85	5,120	KA 13/85	12,800

. Spitz X (10)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 5/85	0	MDH 2/85	1,280
UPM 9/85	640	MDH 10/85	204,800
UPM 31/85	40,960		
UPM 32/85	0	PJ 4/85	640
UPM 53/85	10,240		
UPM 64/85	25,600		
UPM 49/85	5,120		

i. Dobermann X (4)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 34/85	40	UPM 50/85	51,200
UPM 44/85	12,800	UPM 60/85	20,480

i . Labrador X (1)

Serum Sample No.	HI Titre
MDH 5/85	10,240

v Bull Mastiff X (1)

Serum Sample No.	HI Titre
UPM 26/85	320

v . Bull Terrier X (1)

Serum Sample No.	HI Titre
UPM 7/85	2,560

APPENDIX VII

CANINE PARVOVIRUS SERUM HAEMAGGLUTINATION-INHIBITION (HI) ANTIBODY TITRES OF MALE AND FEMALE DOGS

Male

Total number of serum samples = 98 (65%)

Serum Sample No.	HI Titre	Serum Sample No.	HI Titre
UPM 2/85	40	UPM 51/85	20,480
UPM 3/85	6,400	UPM 52/85	10,240
UPM 4/85	10,240	UPM 53/85	10,240
UPM 6/85	80	UPM 54/85	10,240
UPM 8/85	0	UPM 55/85	25,600
UPM 9/85	640	UPM 56/85	25,600
UPM 11/85	1,280	UPM 57/85	51,200
UPM 12/85	160	UPM 59/85	12,800
UPM 13/85	640	UPM 60/85	20,480
UPM 15/85	12,800	UPM 62/85	51,200
UPM 24/85	160	UPM 63/85	10,240
UPM 26/85	320	UPM 65/85	3,200
UPM 28/85	40	UPM 67/85	25,600
UPM 30/85	10		
UPM 31/85	40,960	MDH 6/85	51,200
UPM 34/85	40	MDH 7/85	12,800
UPM 35/85	80		
UPM 36/85	400	PJ 3/85	204,800
UPM 38/85	10,240	PJ 4/85	3,200
UPM 39/85	10,240	PJ 7/85	102,400
UPM 40/85	800	PJ 11/85	1,280
UPM 41/85	20,480	PJ 14/85	3,200
UPM 42/85	6,400	PJ 15/85	5,120
UPM 43/85	520	PJ 17/85	102,400
UPM 45/85	12,800		
UPM 46/85	12,800	KA 1/85	10,240
UPM 48/85	102,400	KA 3/85	10,240
UPM 49/85	5,120	KA 5/85	5,120
UPM 50/85	51,200	KA 6/85	12,800

Serum Sample No.	HI Titre
KA 9/85	102,400
KA 10/85	51,200
KA 11/85	102,400
KA 13/85	12,800
KA 14/85	6,400
KA 15/85	51,200
SP 1/85	51,200
SP 2/85	102,400
SP 4/85	204,800
SP 6/85	204,800
SP 7/85	204,800
SP 8/85	204,800
SP 9/85	102,400
SP 10/85	102,400
SP 11/85	204,800
SP 12/85	102,400
SP 13/85	3,200
SP 14/85	25,600
SP 15/85	3,200
SP 16/85	12,800
SP 17/85	3,200
SP 20/85	12,800
SP 21/85	3,200
SP 22/85	25,600
SP 23/85	25,600
SP 25/85	25,600
SP 26/85	51,200
SP 28/85	12,800
SP 29/85	25,600
SP 30/85	25,600
SP 31/85	3,200
SP 32/85	6,400
SP 33/85	51,200
SP 34/85	6,400
SP 35/85	25,600
SP 36/85	3,200
SP 37/85	25,600
SP 38/85	102,400
SP 39/85	25,600
SP 40/85	6,400
SP 41/85	3,200
SP 43/85	25,600
SP 44/85	6,400

. Female

otal number of serum samples = 53 (35%)

erum Sam le No.	HI Titre
UPM 1/85	10
UPM 5/85	0
UPM 7/85	2,560
UPM 10/85	160
UPM 14/85	160
UPM 16/85	3,200
UPM 17/85	3,200
UPM 18/85	12,800
UPM 19/85	6,400
UPM 20/85	3,200
UPM 21/85	5,120
UPM 22/85	12,800
UPM 23/85	12 ,800
UPM 25/85	320

Serum Sample No.	HI Titre
UPM 27/85	80
UPM 29/85	80
UPM 32/85	0
UPM 33/85	160
UPM 37/85	320
UPM 44/85	12,800
UPM 47/85	25,600
UPM 58/85	5,120
UPM 61/85	3,200
UPM 64/85	25,600
UPM 66/85	25,600
PJ 1/85	25,600
PJ 2/85	25,600
PJ 5/85	5,120
PJ 6/85	6,400
PJ 10/85	5,120
PJ 12/85	2,560
PJ 13/85	25,600
PJ 16/85	12,800

Serum Sample No.	HI Titre
MDH 1/85	160
MDH 2/85	1,280
MDH 3/85	204,800
MDH 4/85	204,800
MDH 5/85	10,240
MDH 8/85	102,400
MDH 9/85	204,800
MDH 10/85	204,800
MDH 11/85	204,800
SP 3/85	102,400
SP 5/85	204,800
SP 18/85	25,600
SP 19/85	102,400
SP 24/85	25,600
SP 27/85	102,400
SP 42/85	51,200
KA 4/85	6,400
KA 7/85	10,240
KA 8/85	25,600
KA 12/85	2,560