Excretion of canine parvovirus type 2 (CPV-2) during gestation and lactation in bitches and puppies

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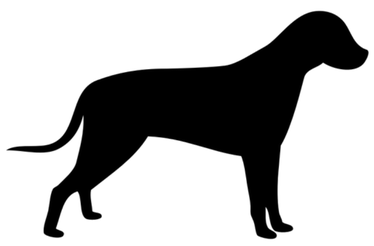
INTRODUCTION

Canine parvovirus type 2 (CPV-2) is a frequent intestinal pathogen associated with high mortality in puppies. Transmission control by disinfection and patient isolation is of limited efficiency, raising questions about other possible contagion sources.

The aim of our study was to determine whether vaccinated dams could excrete CPV2 from mating to end of lactation and so be a potential source of infection for their offspring.

MATERIAL AND METHODS



1 breeding kennel

73 bitches

15 breeds

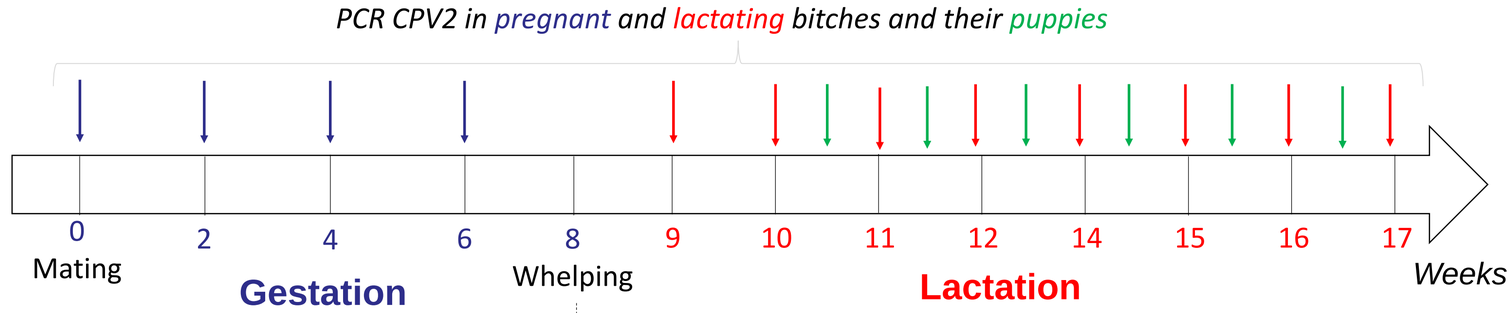
4.4±1.9 years old

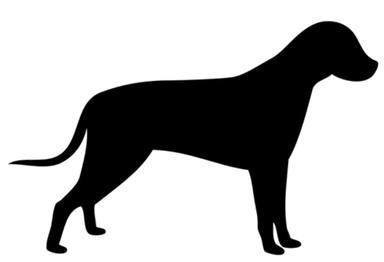
Annually vaccinated (Nobivac DHPPi-Lepto vaccine; MSD)

PCR CPV2

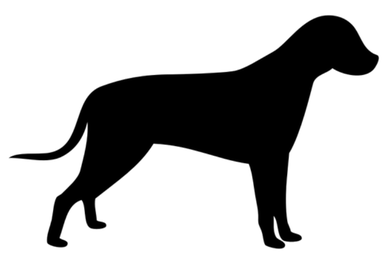
- On **1258** rectal swabs
- **Real time PCR** for CPV-2 genomic DNA (capsid) [1].
- Quantification threshold = **$2x10^5$ copies/g feces** [1]
- Viral load associated with clinical parvovirosis = **$5x10^8$ copies/g feces** [1]

PCR CPV2 in pregnant and lactating bitches and their puppies





41 pregnant bitches

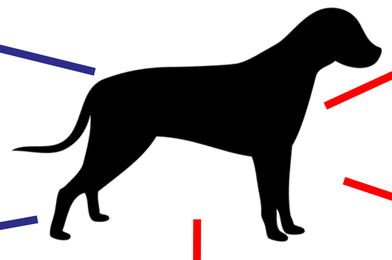


32 lactating bitches

134 puppies

RESULTS

Excretion of parvovirus in adult dogs



Gestation
CPV2 detected in **80%** of bitches at least one time

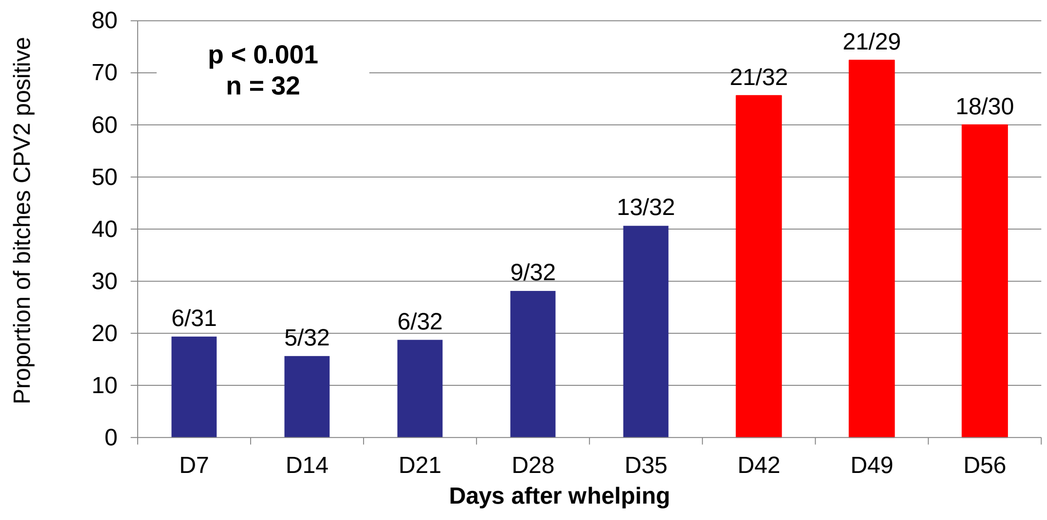
Lactation and **gestation**
No digestive problem observed

Gestation
2% excreting CPV2 at least once above the quantification threshold

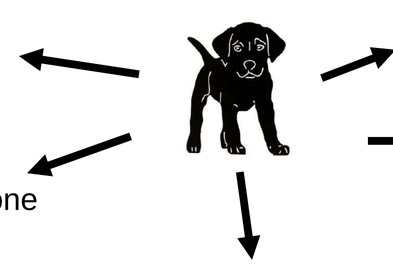
Lactation
64% excreting at least one time above the quantification threshold

Lactation
CPV2 detected in **all bitches** at least one time (Fig.1)

Figure 1: Proportion of bitches CPV2 positive during lactation



Excretion of parvovirus in puppies and relationship with their dams excretion



3% of overall mortality

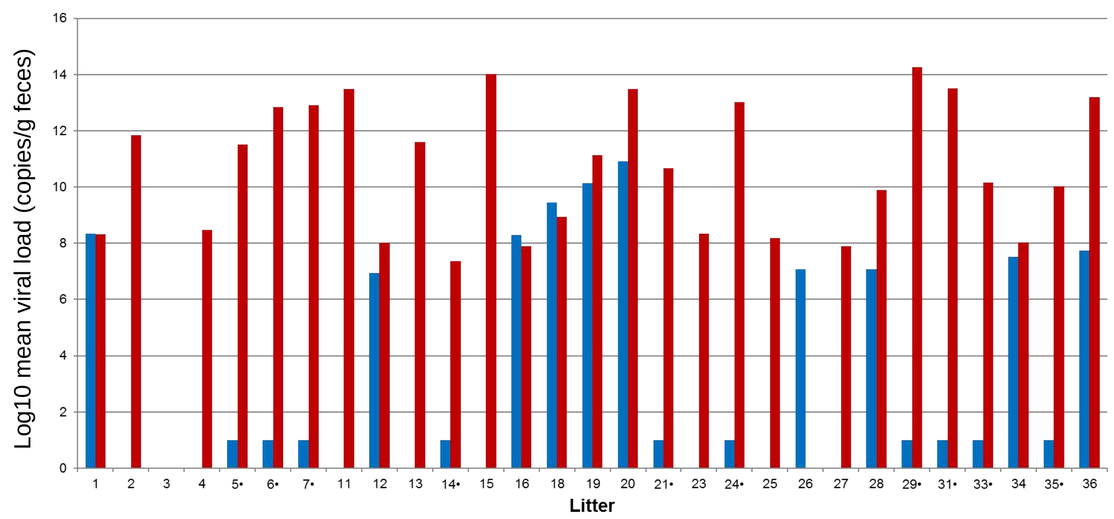
24% started to excrete after their dams

28% started to excrete before their dams

14% experienced at least one episode of diarrhea

Viral loads excreted by puppies were **not correlated** with viral load of their dams (Fig 2).

Figure 2: Mean viral loads excreted by puppies (in red) and their dams (in blue) at 49 days of age



DISCUSSION – CONCLUSIONS

- This study indicates that vaccinated dams may contribute to CPV2 circulation.
- The role of dams in CPV2 circulation in the kennel (between the end of lactation until next whelping) remains to be explored.
- The role of systemic and local immunity in the control of viral excretion and into the control of clinical expression would also be interesting to evaluate, both in dams (after vaccination) and in puppies (quality of the passive immune transfer).

[1] Grellet A, Chastant-Maillard S, Robin C, Feugier A, Boogaerts C, Boucraut-Baralon C, Grandjean D, Polack B. Risk factors of weaning diarrhea in puppies housed inbreeding kennels. Prev Vet Med, 2014. 117(1):260-5.

Steroid hormones in canine X-linked muscular dystrophy using stable isotope dilution liquid chromatography coupled with tandem mass spectrometry

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The Golden Retriever dog is the ideal animal model for preclinical studies of progressive muscular dystrophy, known as Duchenne muscular dystrophy (DMD); this model is referred to as "Golden Retriever muscular dystrophy (GRMD)" [1]. The aim of this study was to determine steroid hormone concentration profiles in healthy Golden Retriever dogs (control group - CtGR) versus GRMD – gene carrier (CaGR) and affected female dogs (AfGR). Therefore, a sensitive and specific analytical method was developed and validated to determine the estradiol (E_2), progesterone (P_4), cortisol, and testosterone levels in the canine serum by isotope dilution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) as previously describe [2]. This multihormone assay was then applied to153 plasma samples collected from 23 female golden retriever dogs throughout their entire estrous cycle (i.e.,anestrous, proestrus,estrus, and diestrus); estrous phase was confirmed by serial vaginal cytology. We observed specific patterns in the concentrations of all hormones that are characteristic of and distinguish the 3 groups across phases of the estrous cycle. It showed statistically significant differences between all groups ($P < 0.05$), except for P_4 and E_2 in the comparison between the CtGR and CaGR groups. The main variable in the separation of the first dimension is E_2; was observed for the AfGR, mean serum levels between 10 to 20 times higher concentration than observed in samples from CtGR and CaGR; irrespective of the phase of the estrous cycle, the mean concentration of E_2 exceeded 1,300pg mL-1 in this group, was different ($P < 0.0001$) compared with the CaGR, and demonstrated discrete variation through the estrous period. Average concentrations of serum cortisol for CaGR and AfGR ranged between1000 and 2000pg mL-1 in all estrous periods, but concentrations were different ($P < 0.05$) between groups during the diestrus. In anestrus and proestrus, P_4 concentrations were similar between groups; but, during estrus and diestrus, concentrations of all quantified hormones were significantly different between the CaGR and AfGR groups. Similar trends were observed for testosterone concentrations during estrus, which were significantly different between the CaGR and AfGR groups ($P < 0.05$). These findings stimulated an important discussion about the correlation of steroid hormones concentrations against clinical signs and development of the pathology of these animals. Data obtained bring new opportunities for hormonal behavior studies in dystrophinopathies and that may affect the quality of life of DMD patients.

[1] Brolio MP , Cima DS, Miglino MA, Ambrosio CE. Histological comparison of the smooth uterine muscle of healthy Golden retriever bitches, carriers of the progressive muscular dystrophy (GRMD) gene, and GRMD-affected bitches. AnimReprodSci.2014;150(10):56–61.

[2] Koala T, Schmiederera D, Pham-Tuana H, Röhringa C, Rauhb M. StandardizedLC–MS/MS based steroid hormone profile-analysis. J Steroid Biochem Mol Biol. 2012;129:129–138.doi:10.1016/j.jsbmb. 2011.12.001PMID:22210511

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Canine parvovirus type 2 (CPV-2) is a frequent intestinal pathogen associated with high pup mortality. Transmission control by disinfection and patient isolation is of limited efficiency, raising questions about other possible contagion sources. The aim of our study was to determine whether vaccinated dams could excrete CPV2 from mating to end of lactation and so be a potential source of infection for their offspring. A total of 73 bitches (mean ± standard deviation: 4.4±1.9 years old; from 15 breeds) housed in the same kennel throughout the reproductive cycle were enrolled in the study. All were annually vaccinated (Nobivac DHPPi-Lepto vaccine; MSD, Beaucouzé, France). Forty-one dams were studied from mating to whelping (Week0-Week8), and 32 dams from whelping until weaning (W9-W16). Rectal swabs were collected every 14 days from dams during gestation (n=41), and every 7 days from lactating dams (n = 32) and their 3-8 week old pups (n=135). Rectal swabs were tested by real time PCR for CPV-2 genomic DNA (capsid) [1]. Using this method, the quantification threshold has been previously established as 2x10^5 copies/g feces and the viral load associated with clinical parvovirosis is 5x10^8 copies/g feces [1]. The number of copies per gram of feces per dog were analyzed through logistic regression and mixed linear models (R, R Foundation for Statistical Computing, Auckland, NZ). Of dams sampled during pregnancy, CPV2 went above the quantification threshold only in one sample vs 64% of dams tested during lactation. During lactation, excreted viral loads were higher at W14 (5x108/g feces, p=0.001), W15 (8x10^8/g feces, p<0.001) and W16 (10^9/g feces, p<0.001) than earlier in lactation (<10^6 copies/g feces; W9-12). None of the bitches, including those excreting viral loads above the threshold for clinical parvovirosis, expressed any clinical sign. In 28% of the cases, the dam excreted before her puppies. Viral loads excreted by puppies were not correlated with those excreted by dams. While the percentage of pups with >5x10^8 CPV2 copies/g feces increased from 2-76% per litter from W10 to W16, the overall mortality was only 3% (4/134) and 14% of the puppies experienced at least one episode of diarrhea. This study indicates that vaccinated dams may contribute to CPV2 circulation. Their role in its persistence in the kennel (between the end of lactation until next whelping) remains to be explored. The role of systemic and local immunity in the control of viral excretion and into the control of clinical expression would also be interesting to evaluate, both in dams and in puppies (quality of the passive immune transfer).

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Sylvie

ABSTRACT BOOK

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