Canine herpesvirus-1 (CHV-1): clinical, serological and virological patterns in breeding colonies

Veerle Ronsse a,*, John Verstegen a, Etienne Thiry b, Karine Onclin a, Christine Aeberle c, Sylvie Brunet c, Hervé Poulet c

a Department of Clinical Sciences, Section Small Animal Reproduction, College of Veterinary Medicine, Université de Liège, Boulevard de Colonster 20, B44, 4000 Liège, Belgium
b Department of Infectious and Parasitic Diseases, Section Virology, Epidemiology and Pathology of Viral Diseases, College of Veterinary Medicine, Université de Liège, Boulevard de Colonster 20, B44, 4000 Liège, Belgium
c Merial, Laboratoire de Lyon Gerland, Lyon, France

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Abstract

Canine herpesvirus-1 (CHV-1) is presumed to be enzootic in the dog population and is associated with reproductive disorders and neonatal mortality. To advise dog breeders towards an effective management of CHV-1 infected colonies, 27 breeding bitches were studied during one reproductive cycle in field conditions: the effect of cycle stage, kennel size, initial antibody titre, mating and gestation on serologic and viral excretion patterns was evaluated, while the association between reproductive disorders and CHV-1 antibody titres and viral excretion was also analysed. All initially seronegative bitches seroconverted, while 40% of the initially seropositive bitches became seronegative at one or two occasions. No difference in antibody patterns was observed between mated and unmated bitches. Of the mated bitches, 46% experienced infertility, foetal resorption or mummification. No difference in antibody patterns was observed depending on the occurrence of reproductive disorders even if a decrease in antibody titres during early or late di-oestrus was often present. Significantly higher titres were observed at all cycle stages in large kennels. None of the vaginal and nasal samples or buffy coats tested positive for CHV-1 DNA. The mixed image of clinical and subclinical carriage in this study demonstrated CHV-1 has a complex and difficult to predict clinical
behavior. Preventive management with vaccination of reproducing bitches in kennels with reproductive disorders should therefore be advised.
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Keywords: Canine; Herpesvirus; Reproductive disorders; Epidemiology

1. Introduction

Canine herpesvirus-1 (CHV-1) was first recognized as an agent responsible for causing a fatal hemorrhagic disease in newborn puppies in 1965 [1]. The virus has been isolated in numerous countries and recent studies in Europe suggest it to be enzootic in the dog population [2–5]. There is some evidence that further to clinical papulovesicular genital lesions [6,7], CHV-1 can also be involved in reproductive disorders such as embryonic resorption, abortion and stillbirth [8–10]. The virus is furthermore associated with respiratory (kennel cough syndrome) and ocular disease in immature and adult dogs [11–13]. Oronasal and venereal transmission are considered to be the main routes of infection, but foetuses can be infected in utero [9,14,15]. As for other alphaherpesviruses, latency in sensory ganglia has been demonstrated for CHV-1 [16,17].

The virus is considered to be poorly immunogenic with neutralizing antibodies disappearing within a few months after infection [15]. Factors altering immunity (such as age, pregnancy, stress, immunosuppressive therapy and concomitant diseases) are determining in herpesvirus pathology [15,18,19].

In a cross-sectional study, we previously demonstrated several factors (a history of kennel cough, the use of external dogs for reproduction, bad hygiene, large kennel size and increasing age) are associated with high CHV-1 antibody titres [22]. Nevertheless, information on longitudinal antibody patterns and frequency of viral, especially vaginal, excretion in natural field conditions remains anecdotal [23]. Published data on longitudinal follow-ups are indeed after experimental CHV-1 inoculation or corticosteroid-induced reactivation [20,21]. Also, viral isolation and seroneutralisation were the tests most commonly used [8,21,23,24]. Few field studies however used the more recently developed and sensitive detection techniques, such as polymerase chain reaction (PCR) and ELISA [16,17].

As CHV-1 is now widespread among the dog population [3–5] and viral excretion and antibody patterns during natural infections are poorly documented, the epidemiological behavior of the virus was investigated prospectively in field conditions. To be able to advise dog breeders towards an effective management of infected colonies, breeding bitches were therefore studied during one reproductive cycle. Differences related to the cycle stage, kennel size, initial antibody titre, the occurrence of mating and pregnancy were investigated. Furthermore, to evaluate the importance of sub-clinical carriage we examined what relationship existed between reproductive disorders and antibody titres or viral excretion. This relationship should indeed indicate the most appropriate breeding and vaccination policy, while present results should also provided information on the value of ELISA and PCR analysis for diagnostic purposes of CHV-1 in field conditions.
2. Materials and methods

2.1. Dog population and sampling

The study group consisted of 27 bitches with a known immune status, originating from 13 different breeding kennels (Table 1) [5]. All bitches were naturally infected and none were vaccinated against CHV-1. Fifteen bitches were mated, 12 were not. Mean age ± S.D. was 4.73 ± 2.82 years. All samples were obtained and examinations performed during one reproductive cycle. For all bitches, sampling was at least performed at the following cycle stages: late anoestrus (5–6 months post last vaginal bleeding), pro-oestrus (vaginal

<table>
<thead>
<tr>
<th>Breeding kennel</th>
<th>Breed</th>
<th>Kennel size</th>
<th>Mated or not mated</th>
<th>Bitch no.</th>
<th>Antibody titre</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Bernese Mountain dog</td>
<td>3</td>
<td>Mated</td>
<td>1</td>
<td>2.13</td>
</tr>
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<td></td>
<td></td>
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<td>2</td>
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<td></td>
<td></td>
<td>3</td>
<td>2.65</td>
</tr>
<tr>
<td>2</td>
<td>Irish Wolfhound</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>2</td>
<td>English Setter</td>
<td>2</td>
<td>Not mated</td>
<td>4</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>5</td>
<td>1.99</td>
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<td>7</td>
<td>1.91</td>
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<tr>
<td>3</td>
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<td>Mated</td>
<td>8</td>
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<td>9</td>
<td>1.77</td>
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<tr>
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<td>English Cocker Spaniel</td>
<td>3</td>
<td>Not mated</td>
<td>10</td>
<td>1.47</td>
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<tr>
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<td>1</td>
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<td>16</td>
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<tr>
<td>8</td>
<td>Irish Wolfhound</td>
<td>2</td>
<td>Mated</td>
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<tr>
<td>9</td>
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<td>21</td>
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<td></td>
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<td></td>
<td>22</td>
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</tr>
<tr>
<td>12</td>
<td>English Bulldog</td>
<td>2</td>
<td>Mated</td>
<td>23</td>
<td>0.48</td>
</tr>
<tr>
<td>13</td>
<td>Beagle</td>
<td>3</td>
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<td>24</td>
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<td>27</td>
<td>1.78</td>
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</tbody>
</table>

a (1) Less than 6 dogs; (2) 6–20 dogs; (3) more than 20 dogs.
b Immune status 6–8 months prior to the start of the study.
bleeding and progesterone values $< 2$ ng/ml), oestrus (progesterone values between 2 and 20 ng/ml), early di-oestrus (25–30 days post-estimated LH-surge), late di-oestrus (55–63 days post-estimated LH-surge) and early anoestrus (78–87 days post-estimated LH surge). In some bitches, more samples were collected during these cycle stages and at metoestrus rush (vaginal cytology after Johnston et al. [25] and progesterone $> 20$ ng/ml) and mid-di-oestrus (35–50 days post-estimated LH surge). Overall, 266 consultations and samplings were performed.

At each sampling, blood was collected by jugular venepuncture. Five milliliters of blood were put into plain glass tubes and another 5 ml into heparinized tubes. All blood samples were centrifuged at 3500 rpm/min for 15 min. Serum and plasma were collected and stored in two times 1 ml aliquots at $-80^\circ$C until analysis. Buffy coats were collected from 86 samples and stored in two times 1 ml aliquots at $-80^\circ$C. At each sampling, vaginal and nasal swabs were simultaneously collected and plunged in two times 1 ml aliquots with 0.5 ml physiologic fluid. Aliquots were kept at $-80^\circ$C until analysis.

2.2. Clinical examination and history

For determining cycle stage during heat, vaginal cytology and vaginoscopy were performed and evaluated as described by Johnston et al. [25,26]. Vulva and vestibulum vaginae were checked for CHV-1 associated lesions at all cycle stages. For the investigated cycle, history of the following items was obtained:

1. Date of mating.
2. For mated bitches:
   - Abortion, dystocia or section.
   - Number of puppies at birth and neonatal mortality within the first 3 weeks of life.

2.3. Ultrasonography

During early and late di-oestrus, uterin ultrasonography was performed on mated bitches. During early di-oestrus number of foetuses and occurrence of foetal resorption were recorded. During late di-oestrus number of foetuses, decreased foetal viability (foetal heart rate $< 200$ beats/min) and mummiﬁcation were recorded.

2.4. Progesterone analyses

Concentrations were measured with a commercial radioimmunoassay kit (Progesterone Coat-a-count Kit: Diagnostic Products Corporation, Los Angeles, CA, US), validated for dog plasma [27,28]. The sensitivity at 95% bound was 0.3 nmol l$^{-1}$ and the intra- and interassay coefficients of variation were 5.1 and 8.8%, respectively. The day of the LH peak was estimated to be the day when progesterone concentrations reached values between 2.5 and 10 nmol l$^{-1}$ [29].
2.5. Antibody titration

All serum samples were analysed for CHV-1-specific antibodies with a previously described and validated ELISA [5]. All sera from a single animal were tested in the same session. Antibody titres were expressed as log_{10} values and classified into four categories: negative (<1), weakly positive (1–1.5), moderately positive (1.51–2) and strongly positive (>2). For analyses, all titres inferior to 0.48 were assigned to an arbitrary fixed value of 0.48. Bitches included in the study had ELISA antibody titres ranging from <0.48 (negative) to 2.65 (strongly positive). Repeatability standard deviation was 0.05.

2.6. DNA extraction and PCR analysis

2.6.1. DNA extractions

DNA from vaginal and nasal samples was extracted with iminodiacetic acid chelating resin (Chelex 100, Sigma, St. Louis, US) and Nonidet P 40 (Fluka Chemie GmbH, Buchs, Switzerland). Extraction was performed by adding 100 µl of 0.2% Nonidet P 40 and 0.2 g of chelating resin to 100 µl of thawed aliquot [30]. Further dilution was performed with 200 µl ultrapure Dnase, Rnase free water. After 15 s of vortexing, the mixture was boiled for 10–15 min and kept on ice until PCR analysis was performed. Extracted products were analysed with PCR amplifying a section of the gene encoding for the viral glycoprotein B (gB). Some samples were also extracted with a commercial extraction kit (Dneasy Tissue Kit, Qiagen, Courtaboeuf, France) after the manufacturer’s recommendations and subsequently analysed with a PCR amplifying a section of the gene encoding for the glycoprotein D (gD). Buffy coats were extracted with the same commercial extraction kit (Dneasy Tissue Kit, Qiagen) and extracted products were analysed with the PCR amplifying a section of the gene encoding for the viral gB.

2.6.2. PCR analysis amplifying a section of the gene encoding for gB

A section of 450 bp of the CHV-1 gB was amplified using an upstream primer of 20 bp (5’-CCTAAACCTACTTCGGATGA-3’) and a downstream primer of 22 bp (5’-GGCTTTAAATGAACCTTCTCTGG-3’) (Eurogentec, France). The PCR reaction was performed using a Biometra Gradient 96 DNA or Perkin-Elmer 480 PE thermal cycler. A commercial preparation (Hot Star Taq Mastermix Kit, Qiagen, Courtaboeuf, France) was used with the following final concentrations of reagents: 1.2 mM MgCl₂, 200 µM of each dNTP, 1.25 U Taq polymerase, 0.2 µM upstream and 0.2 µM downstream primer. Six microliters of extracted sample were used in a final reaction volume of 30 µl. In each PCR, positive and negative controls were included. For a positive control, supernatant of CHV-1 F-205 viral culture was used. For a negative control, ultrapure Dnase and Rnase free water was used. Viral DNA was amplified by an initial denaturation step at 94 °C for 3 min followed by 60 cycles of denaturation at 94 °C for 50 s, annealing at 49 °C for 50 s and synthesis at 72 °C for 50 s. An extension time of 10 min was added at the end of the final cycle.
2.6.3. **PCR analysis amplifying a section of the gene encoding for gD**

The PCR analysis amplifying a section of 548 bp of the gene encoding for gD was performed as previously described and validated [31].

Specificity and sensitivity of both PCR analyses were validated [32].

2.7. **Gel electrophoresis**

For gel electrophoresis, 10 μl PCR product was used on a 0.8% agarose gel prepared with a Tris/acetic acid buffer (24.5% (p/v) Tris, 5.71% (v/v) acetic acid, 1.86% (p/v) EDTA). Gel electrophoresis was carried out at 100 V/cm for 15 min. Gels were incubated with ethidium bromide for 15 min and photographed under UV illumination at 312 nm. The size of PCR products was evaluated by comparison with a DNA standard size (Smartladder SF, 100–1000 bp ladder, Eurogentec, San Diego, US).

2.8. **Statistical analysis**

Statistic Analysis System (SAS) was used for statistical analysis [33]. Statistical analyses were performed on the six in common cycle stages. For evaluating differences in antibody titres between cycle stages, general linear models (proc glm) were used. For evaluating differences in antibody titres between mated and unmated bitches and for comparison of antibody titres between mated bitches with and without infertility or reproductive disorders, the Wilcoxon Mann–Whitney U-test (proc npar1way) was used. Analyses of antibody titres depending on the titre at the start of the trial and depending on kennel size were performed using the Kruskal–Wallis analysis of variance test (proc npar1way). For comparison of infertility or reproduction disorders depending on age and kennel size, Fisher’s Exact test was used (proc freq). Differences were considered significant when $p < 0.05$.

3. **Results**

3.1. **Serology and reproductive disorders**

When comparing mean antibody titres between cycle stages, no significant differences could be observed in the whole population (Fig. 1), although a weak decrease in antibody titres was observed during di-oestrus. Most bitches were seropositive at the start of the trial. Of those, 15 remained seropositive at all stages, while 5 became seronegative at one occasion and 5 at two occasions. Again, the most dramatic drops in antibody titres (from 1.1–1.3 to <0.48) were observed in di-oestrus. The two bitches that were seronegative at the start of the trial seroconverted during oestrus (Fig. 2). Overall, four bitches had an increase in their antibody titre at the time of oestrus. In three of them, titres remained stable during di-oestrus and declined in anoestrus, whereas titres dropped in early di-oestrus for the fourth bitch. This bitch suffered partial embryonic resorption.

When comparing antibody titres depending on the initial titre at the start of the trial (late anoestrus), significantly higher titres were observed during all cycle stages for bitches with
an initial titre superior to 2 compared to bitches with an initial titre between 1 and 2: late anoestrus ($p < 0.0001$), pro-oestrus ($p < 0.0012$), oestrus ($p < 0.0009$), early di-oestrus ($p < 0.0004$), late di-oestrus ($p < 0.0038$) and early anoestrus ($p < 0.0064$). Furthermore, significantly higher titres were observed during late anoestrus ($p < 0.0013$) and pro-oestrus ($p < 0.0018$) in bitches with an initial titre between 1 and 2 compared to bitches with an initial titre superior to 2. Significantly higher titres were observed in late anoestrus, early and late di-oestrus and early anoestrus in bitches with an initial titre superior to 2 compared to bitches with an initial titre inferior to 1.

No significant differences in mean antibody titres were observed between mated and unmated bitches (Fig. 3). Infertility (no pups after mating) and reproductive disorders occurred in 7 out of the 15 (47%) mated bitches. Infertility was encountered on three occasions, while reproductive disorders consisted in two bitches with partial foetal resorptions and two with mummifications. Two types of antibody patterns were observed in these bitches: either a considerable decrease in titres (more than 0.5) was present during
early di-oestrus, either continuously positive titres were present with a decrease in late di-oestrus.

Vaginal lesions or neonatal mortality possibly related to CHV-1 were not observed. No significant differences in mean antibody titres were observed between mated bitches with and without reproductive disorders, although Fig. 4 shows a tendency towards higher titres in oestrus and early di-oestrus in bitches without reproductive disorders. No significant differences in reproductive disorders were observed depending on age (Table 2), although a tendency ($p < 0.13$) towards a higher frequency of reproductive disorders was observed in bitches of 5 years and older. When comparing mean antibody titres in kennels of different sizes, significantly higher titres ($p < 0.001$) were observed in kennels with more than 20 dogs than in kennels with less than 6 dogs and 6–20 dogs (Fig. 5). Differences were significant at all cycle stages: late anoestrus ($p < 0.004$), pro-oestrus ($p < 0.033$), oestrus ($p < 0.0049$), early di-oestrus ($p < 0.0065$), late di-oestrus ($p < 0.01$) and early anoestrus ($p < 0.01$). No significant differences were observed between kennels with less than 6 dogs and 6–20 dogs. No difference in occurrence of infertility or reproductive disorders was observed depending on kennel size.

Fig. 3. Mean antibody titres ± S.E.M. for mated and unmated bitches. No significant differences were observed.

Fig. 4. Mean antibody titres ± S.E.M. depending on the occurrence of reproductive disorders. No significant differences were observed.
Table 2

Observed frequencies of infertility and reproductive disorders depending on age

<table>
<thead>
<tr>
<th>Age</th>
<th>Infertility or reproductive disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>6</td>
</tr>
<tr>
<td>≥5 years</td>
<td>2</td>
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</tbody>
</table>

No significant differences were observed.

Fig. 5. Mean antibody titres ± S.E.M. depending on kennel size. Significantly higher titres were observed in kennels with more than 20 dogs than in kennels with less than 6 and 6–20 dogs. No significant differences were observed between kennels with less than 6 and 6–20 dogs.

**PCR gB**

Fig. 6. Results of PCR amplifying a section of the gene encoding for gB (top) and gD (bottom). Positive control bands are present in lane 11, negative control bands in lane 12.
3.2. **PCR analyses**

All vaginal and nasal samples tested negative on PCR gB and gD analyses (Fig. 6). Positive control bands are present in lane 11, negative control bands in lane 12. All buffy coats were also found negative with gB PCR analysis.

4. **Discussion**

This is the first prospective study monitoring CHV-1 antibody patterns, nasal and vaginal viral excretion as well as viraemia in parallel in naturally infected dog breeding colonies.

The use of a sensitive and highly reproducible ELISA made it possible to study antibody kinetics and detect mild fluctuations. In a few bitches with frequent serum sampling, reproducibility of the ELISA was confirmed by comparing subsequent samples. Fluctuations were usually lower than 0.1.

In this study, all initially seronegative dogs seroconverted during the investigated reproductive cycle. However, while 60% of those seropositive dogs remained seropositive, 40% became seronegative on one or two occasions. In fact, these fast serologic changes together with the existence of latency for CHV-1 [16,17] suggest that in many breeding colonies, the entire dog population is infected.

The virus is also presumed to be poorly immunogenic [15]. Although this presumption could be related to the use of antibody detection techniques with a low sensitivity (such as the serum neutralization test), our study showed that when using highly sensitive CHV-1 antibody detection techniques (such as the ELISA), rapid serologic changes may indeed occur. Our results illustrate the need for repeated serologic testing when using an ELISA, since testing at a single time point will not always inform correctly on the infection status of the animal. The overall prevalence of CHV-1 in the dog population is therefore probably underestimated.

Furthermore, the degree of seropositivity among bitches may vary from one kennel to another. Appel [23] mentioned seroprevalences of 20–90% in CHV-1 affected kennels. As we previously demonstrated [22], individual and environmental factors on the kennel level could explain these differences among kennels. Higher seroprevalences are indeed expected in larger kennels, especially if bad hygienic conditions and a history of kennel cough are present [22]. More specific results were obtained in this trial. In fact, although in certain bitches rapid serologic changes were observed, CHV-1 antibody titres sometimes persisted elevated for several months, especially in kennels with more than 20 dogs. This persistence of moderately or strongly positive titres shows that CHV-1 spreading through recurrent nasal excretions is more frequent in large kennels. Appel [23] also mentioned the existence of nasal periodic CHV-1 shedders. Recurrent excretion at the kennel level (by one or different individuals at different stages) could indeed explain why in this study we were able to observe small to moderate boosts in antibody titres in certain bitches (data not shown). Moreover, it can be notified that these boosts could only be observed in bitches that were sampled on frequent occasions, again demonstrating the importance of repeated serological testing. The importance of nasal transmission in kennels was also reflected by the similar antibody patterns observed in mated and unmated bitches. However, according
to our previous observations [22], CHV-1 is probably transmitted by oronasal, genital and maybe orogenital route in kennels.

Although there was no significant difference in mean antibody titres between cycle stages, antibody titres were usually lower during di-oestrus. This absence of significance could be related to the heterogeneity of the population in this trial, including bitches with persistent high titres. In women, it was indeed demonstrated that the stage of pregnancy affects herpes simplex virus type-2 (HSV-2) antibody titres with lower titres in advanced stages of pregnancy [34]. In kennels, other important parameters, like exposure to respiratory aerosols of infected dogs, may affect CHV-1 antibody titres and interfere with potential fluctuations related to the cycle stage. In fact, di-oestrus might be a critical period associated with a low anti-CHV-1 immunity if one considers that CHV-1 antibodies reflect the general immune status against the virus. Di-oestrus was also shown to be a critical period for HSV-2 infection in mice models [35].

Elsewhere, CHV-1 has been demonstrated to cause abortion during the second and third trimester of gestation and neonatal mortality in pups until the age of 3 weeks. Its importance in reproductive disorders in earlier stages of gestation (embryonic resorption and bitches remaining ‘empty’ after mating) has been suggested by different authors [8,31], but could not be demonstrated up to now because of the lack of diagnostic methods during the first trimester of gestation in this species [36]. The implication of herpesvirus in early pregnancy has been demonstrated in studies on heifers infected with bovine herpesvirus-1 and mice with HSV-2 [37,38]. The relationship between the serological status of a bitch and in utero clinical manifestation however appears controversial. Some authors suggest no relationship exists, while in a previous study we demonstrated seropositive bitches more commonly have a history of abortion [22,23]. In this trial, some bitches with fertility or reproductive disorders had moderately to strongly positive titres throughout the investigated reproductive cycle, while others with a considerable decrease in antibody titres in early or late di-oestrus, often experienced infertility or reproductive disorders (foetal resorption or mumification). This last observation was consistent with the results of Poulet et al. [31] who demonstrated in a vaccination trial higher pregnancy rates (82% versus 67.9%) were observed in bitches vaccinated 10 days after mating and again 6 weeks later. These results suggest high antibody titres in oestrus and early di-oestrus may be indicative of a protective immune response against in utero clinical signs (resorption, abortion, mumification). However, as in our study reproductive disorders were also observed in bitches with high antibody titres, nasal excretion could have accounted for these titres. In those cases, reproductive disorders were not related to CHV-1 or CHV-1 in utero infection was a local phenomenon. It can indeed be suggested that not only humoral, but also cell-mediated immunity is important in CHV-1 in utero pathogenesis. This was already demonstrated for CHV-1 infection in neonatal puppies, but not in bitches of reproductive potential [15]. However, exact in utero pathogenesis after reactivation remains unknown. As CHV-1 latency has been demonstrated in the lumbosacral ganglion, it cannot be excluded that in utero infection after reactivation appears without viremia [16,17]. No viral DNA could indeed be detected in white blood cells in this trial. It should moreover be mentioned that for cases of infertility, other causes than CHV-1 (such as bad breeding management, other infectious agents and inbreeding) may be responsible for this outcome. Possibilities of that were however reduced by performing clinical follow-ups during heat and gestation and by advising dog breeders towards a correct breeding management.
The effect of age on reproduction disorders was not statistically significant. However, the observed tendency towards more infertility and reproductive disorders in bitches older than 5 years as well as the author’s experience suggest this may be related to the limited number of bitches in this study. It is indeed known factors affecting immunity and general condition may influence herpesvirus pathology.

No nasal and vaginal samples were positive on PCR analysis. Previously, other authors already mentioned the difficulty of isolating CHV-1 when using viral isolation, and this as well during “quiescent periods” [8,23] as when lesions were present [7]. Vaginal shedding was occasionally detected using the CHV-1 gB PCR in a contaminated kennel and gB PCR was confirmed to be more sensitive than virus isolation [32]. Results in this study however showed that even when using the more sensitive PCR, negative results should be interpreted very carefully, as in natural field conditions where CHV-1 is endemic and immunity is present, viral excretion is presumably brief and only lasts for a few days. In humans, asymptomatic shedding of HSV-2 is indeed shorter than symptomatic shedding and may last for only a single day [39]. None of the bitches included in this study had genital lesions, thereby reducing the probability to detect CHV-1.

Our results also have implications for antibody and PCR testing as a diagnostic tool: because of the sudden decrease in antibody titres observed in certain bitches, single antibody testing appears a poor indicator for CHV-1 infection. To identify the presence of CHV-1 in kennels, it is preferable to sample several dogs at the same time or the same dog repeatedly. As we failed to demonstrate viral DNA in vaginal and nasal excretions, PCR analysis, used for diagnostic purposes, should be realized at specific times preferentially when reproductive disorders or genital lesions occur. Also, as excretion may be very short, it can be advised to sample dogs on consecutive days. For the same reasons, the use of PCR at mating (as a preventive measure) seems a rather useless tool, certainly if clinical signs are absent.

Furthermore, although CHV-1 is most commonly associated with neonatal mortality, all reproduction disorders encountered in this trial occurred during gestation. The majority of bitches indeed had positive titres at whelping and neonatal mortality is often mentioned to occur primarily at primo-infection [15,23,40]. In breeding units, where CHV-1 is endemic and immunity is present, latency-associated pathogenesis could be different than at primo-infection, especially for reproduction disorders occurring in utero. Results from this trial therefore indicate reactivation associated with in utero pathogenesis should be further investigated.

In conclusion, the mixed image of clinical and sub-clinical carriage demonstrated CHV-1 has a complex and difficult to predict clinical behavior. Antibody titres are influenced by fluctuations related to the estrous cycle and to possible external contaminations. As natural antibody patterns appear unpredictable, a preventive management with vaccination of reproducing bitches in kennels with a history of reproductive disorders should be advised.

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References


