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Mariarosaria Marinaro, Anna Lucia Bellacicco, Michele Camero, Elvira Tarsitano, Maria Tempesta, Antonio Cassone, Canio Buonavoglia

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1 **Caprine herpesvirus-1-specific IgG subclasses in naturally and experimentally**  
2 **infected goats**

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6 Mariarosaria Marinaro<sup>°\*</sup>, Anna Lucia Bellacicco, Michele Camero, Elvira Tarsitano, Maria  
7 Tempesta, Antonio Cassone<sup>°</sup>, Canio Buonavoglia

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10 <sup>°</sup>Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di  
11 Sanità, Rome, Italy

12 Department of Veterinary Public Health, Università degli Studi di Bari, Bari, Italy.

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16 \*Corresponding author:

17 Mariarosaria Marinaro, Ph.D.

18 Department of Infectious, Parasitic and Immune-mediated Diseases

19 Istituto Superiore di Sanità

20 Viale Regina Elena 299

21 00161 Rome, Italy

22 mary\_marinaro@libero.it

23 Fax +39.080.4679.843

24 Tel.+39.080.4679.841

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27 **Running head:** caprine herpesvirus-specific IgG subclasses

1 **Abstract**

2 Enzyme-linked immunosorbent assays (ELISAs) were developed to detect caprine  
3 herpesvirus-1 (CpHV-1)-specific IgG1 and IgG2 in sera from 43 naturally-infected goats.  
4 The analysis of the IgG subclasses showed a dual pattern of distribution in seropositive  
5 goats with a major group of animals (36 out of 43) exhibiting significantly higher levels of  
6 IgG2 over IgG1 and a minor group (7 out of 43) possessing equal levels of IgG1 and IgG2.  
7 Four goats were experimentally infected with a virulent CpHV-1 Ba.1 strain by the  
8 intranasal or the intravaginal route and the kinetics of appearance of CpHV-1-specific IgG,  
9 IgG1 and IgG2 in the serum were studied. Two weeks following infection, both IgG1 and  
10 IgG2 levels increased although convalescent sera (i.e., collected five to eight weeks post-  
11 infection) showed a clear prevalence of the IgG2 subclass. To determine the contribution of  
12 the different IgG subclasses to herpesvirus immunity, serum neutralization (SN) assays  
13 were performed in both naturally and experimentally infected goats. The kinetics of SN  
14 showed that neutralization activity was mainly associated to the IgG1 subclass and this  
15 was also confirmed in naturally infected goats. The results are discussed from the  
16 standpoint that the profile of the IgG subclasses is instrumental to study immune responses  
17 to CpHV-1 and that vaccination strategies may benefit from this information.

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23 *Keywords:* CpHV-1; IgG subclass; viral neutralization; IgG; goat immunity

## 1 1. INTRODUCTION

2 Caprine herpesvirus 1 (CpHV-1) belongs to the *Herpesvirales* order, *Herpesviridae* family,  
3 *Alphaherpesvirinae* subfamily, *Varicellovirus* genus (Davison et al., 2009) and causes two  
4 different syndromes depending on the age of the animal at the time of infection. In adult  
5 goats, the infection is often subclinical or it results in vulvovaginitis, balanoposthitis or  
6 spontaneous abortion (Grewal and Wells, 1986; Saito et al., 1974; Tarigan et al., 1987;  
7 Williams et al., 1997). In one- or two-week old kids, CpHV-1 is responsible for a systemic  
8 disease characterized by high morbidity and mortality, and ulcerative and necrotic lesions  
9 throughout the enteric tract (Mettler et al., 1979; Van der Lugt and Randles, 1993). CpHV-  
10 1 enters the animals via the genital or the respiratory tract and establishes latent infection  
11 in the sacral and trigeminal ganglia (Tempesta et al., 1999b). In natural infection, it can  
12 reactivate during the mating season probably as a result of the stress associated to hormonal  
13 changes (Tempesta et al., 1998) while in experimental infection, reactivation can be  
14 achieved following administration of high doses of dexamethasone (Buonavoglia et al.,  
15 1996). CpHV-1 infection is distributed worldwide although it is responsible for major  
16 economical losses in Mediterranean countries (Thiry et al., 2006). The CpHV-1 shares  
17 many biological similarities with human herpesvirus 2 (HHV-2) and although many  
18 reports have shown that mice could be used to study HHV-2 infection (reviewed in  
19 Ferenczy, 2009) others have shown that the CpHV-1/goat is a useful model to study HHV-  
20 2 infection in humans (Tempesta et al., 1999a; 1999b; 2000; 2007c). Thus, implementation  
21 of the studies on CpHV-1 will also provide benefit to human medicine where vaccines  
22 against HHV-2 are urgently needed (Ferenczy, 2007). Although some data has  
23 accumulated on the pathogenesis and latency of CpHV-1 infection, there is limited

1 information on the immune responses to CpHV-1 (Tempesta et al., 2005; 2007b) and no  
2 information is available on IgG subclasses.

3 Cattle have three transcriptionally active genes encoding three IgG subclasses (Rabbani et  
4 al., 1997), namely IgG1, IgG2, and IgG3; sheep and goats also seem to have the same  
5 genes (Butler, 1998; Micusan and Borduas, 1977) but the lack of commercially available  
6 antibodies specific to IgG3 dampens the elucidation of the functional role of the third IgG  
7 subclass in these ruminants.

8 Caprine IgG1 and IgG2 responses have been studied by western blot analysis (employing  
9 cross-reactive anti-bovine IgG subclass antibodies) following genetic vaccination with a  
10 caprine arthritis-encephalitis virus envelope gene (Cheevers et al., 2000) and in  
11 *Chlamydophila psittaci* infection where it is important to determine the profile of the IgG  
12 subclass in order to distinguish clinical inapparent infections from overt disease (Schmeer  
13 et al., 1987). The aim of the present study was to evaluate the distribution of CpHV-1-  
14 specific IgG1 and IgG2 in caprine herpesvirus infection by developing CpHV-1-specific  
15 ELISAs. In addition, the contribution of the IgG subclasses to the neutralization activity of  
16 sera from naturally and experimentally infected goats was also studied.

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## 1 2. MATERIALS AND METHODS

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### 3 2.1. Animals

4 To select the naturally-infected goats, 63 goats of mixed breed, age (range 1-4 years) and  
5 sex (10 males, 53 females) were screened for serum IgG by using a CpHV-1-specific  
6 ELISA. Among the 63 animals screened, there were 20 seronegatives and 43 seropositives;  
7 the latter were used for the subsequent evaluation of serum CpHV-1-specific IgG  
8 subclasses. For the experimental infection, four additional goats (females, age 3-4 years)  
9 were employed. All the animals included in the study were free of caprine arthritis-  
10 encephalitis virus. Blood samples were aseptically obtained from the jugular vein and  
11 serum was collected by centrifugation at 2000 rpm for 10 min (Beckman microfuge,  
12 Fullerton, USA). Serum samples were stored at  $-20^{\circ}$  C until tested.

13

### 14 2.2. Virus

15 The Ba.1 strain of CpHV-1 was used to experimentally infect the animals or to prepare the  
16 antigen for the ELISA (Buonavoglia et al., 1996). The virus was propagated by infecting  
17 Madin Darby Bovine Kidney cells (MDBK; ATCC-LGC Standards, Milan, Italy) grown on  
18 Dulbecco-Minimal Essential Medium (D-MEM; Lonza, Walkersville, USA) supplemented  
19 with 10% foetal calf serum (FCS; Lonza). The viral titer was  $10^{7.00}$  50% tissue culture  
20 infectious doses (TCID<sub>50</sub>)/50 $\mu$ l. The viral suspension was tested for bacterial or fungal  
21 contamination.

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### 2 **2.3. Experimental infections**

3 For the intravaginal infection, two goats received 4 ml of CpHV-1 Ba.1 strain ( $10^{7.00}$   
4  $\text{TCID}_{50}/50\mu\text{l}$ ) in the vagina. For the intranasal infection, two goats received 2 ml per nostril  
5 of the same viral suspension as described above. Goats were kept under observation for two  
6 months and examined daily for general and clinical signs, including body temperature, as  
7 described previously (Tempesta et al., 2000; 2007a; 2007b; 2007c). At the indicated time  
8 points, goats were bled and sera were collected and stored at  $-20^{\circ}\text{C}$  until tested by ELISA  
9 and neutralization assays.

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### 11 **2.4. Elisa for serum IgG, IgG1 and IgG2**

12 To measure CpHV-1-specific IgG responses, an assay in use in our laboratory was  
13 employed (unpublished). In particular, 96-well polystyrene plates (Nunc, Roskilde,  
14 Denmark) were coated with  $100\ \mu\text{l}/\text{well}$  of CpHV-1 (total protein content,  $25\ \mu\text{g}/\text{ml}$ ) in  
15 carbonated buffer ( $15\text{mM Na}_2\text{CO}_3$ ,  $35\ \text{nM NaHCO}_3$ , [pH 9,6]) and incubated overnight at  
16  $4^{\circ}\text{C}$  on a shaker. After blocking the wells with  $200\ \mu\text{l}$  of 2 % gelatin/PBS (Sigma, Milan,  
17 Italy) for 2 hours at  $37^{\circ}\text{C}$ , individual serum samples diluted 1:100 in 0.05% Tween 20/PBS  
18 (PBS/T) were added to duplicated wells and incubated overnight at  $4^{\circ}\text{C}$ . After washing,  
19 HRP-conjugated rabbit anti-goat IgG (Bethyl, Montgomery, USA) diluted 1:1000 in PBS/T  
20 were added to the wells and incubated for 2 hours at  $37^{\circ}\text{C}$ . After final washings and  
21 addition of ABTS, the colorimetric reaction was measured at 405 nm with an ELISA plate  
22 reader (Biorad, Hercules, USA). The O.D. values were recorded and individual readings

1 were reported by subtracting the O.D. values of negative controls (i.e., negative serum  
2 samples) to the individual O.D. values. All serum samples were run under identical  
3 conditions and, where possible, on the same microtiter plate. The O.D of negative control  
4 sera or background wells (without serum) was 0.030-0.050.

5 To measure IgG subclasses, a protocol similar to that employed for total IgG was employed  
6 with some modifications: a) serum samples were diluted 1:10 and incubated overnight at  
7 4° C; b) HRP-conjugated sheep anti-bovine IgG1 (diluted 1:100 in PBS/T) or HRP-  
8 conjugated sheep anti-bovine IgG2 (diluted 1:50 in PBS/T) were employed as detection  
9 antibodies (Bethyl); c) the latter detection antibodies were incubated overnight at 4° C. The  
10 O.D. values (read at 405 nm) were recorded and individual readings were reported by  
11 subtracting the O.D. values of negative controls (i.e., negative serum samples) to the  
12 individual O.D. values. All serum samples were run under identical conditions and, where  
13 possible, on the same microtiter plate. The O.D of negative control sera or background  
14 wells (without serum) was 0.040-0.050 for IgG1 and 0.045-0.055 for IgG2. Preliminary  
15 experiments established that sheep anti-bovine IgG1 or IgG2 cross-reacted with goat IgG  
16 subclasses. In addition, we performed serum dilution curves with several sera exhibiting a  
17 different ratio IgG1:IgG2; over a large serum dilution range, the curves generated with the  
18 anti-IgG1 and the anti-IgG2 were parallel to each other (not shown). The distribution of IgG  
19 subclasses was not affected by breed, age or sex.

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## 21 **2.5. SN assays**

22 Serum neutralization assays were performed as described elsewhere (Tempesta et al.,  
23 2000). Briefly, sera were heat inactivated at 56° C for 30 min and serial two-fold dilutions

1 were mixed with 100 TCID<sub>50</sub> of CpHV-1 Ba.1 strain in 96 well flat bottom plates (NUNC).  
2 Plates were incubated at room temperature for 90 min and then 20000 MDBK cells were  
3 added to each well. Following three days of incubation at 37°C with 5% CO<sub>2</sub>, the endpoint  
4 titers were determined as the highest serum dilution which neutralized the cytopathic effect  
5 of the virus.

6

## 7 **2.6. Statistical analyses**

8 Comparison of data was performed by using Student's *t*-test or ANOVA (when variance  
9 between groups was different) and is indicated in the figure legends. A *p* value less than  
10 0.05 was considered significant.

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### 1 3. RESULTS

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#### 3 3.1. Distribution of CpHV-1-specific serum IgG1 and IgG2 in naturally infected 4 goats.

5 To select the seropositive animals, 63 goats were initially screened for CpHV-1-specific  
6 serum IgG by using an ELISA. Figure 1 shows the CpHV-1-specific IgG levels in the 63  
7 goats. Forty-three seropositive animals were identified and were subsequently employed for  
8 the evaluation of the IgG subclasses, while the 20 seronegative goats were excluded from  
9 the analysis. When the 43 positive sera were subjected to the CpHV-1-specific IgG1 and  
10 IgG2 ELISAs, the distribution of the IgG1 and IgG2 segregated into two different profiles.  
11 Figure 2A shows the distribution of CpHV-1-specific IgG1 and IgG2 in a major group of  
12 animals, i.e. 36 goats out of 43, referred to as Group 1. These animals exhibited IgG2  
13 levels significantly higher than IgG1 ( $p < 0.05$ ). A minor group of animals, i.e. 7 goats out  
14 of 43, possessed a different distribution of IgG1 and IgG2 than Group 1. This minor group  
15 was referred to as Group 2, and included all the animals possessing equal levels of IgG1  
16 and IgG2 in their sera (Fig. 2B). Since the differences between Group 1 and Group 2  
17 seemed not solely qualitative but also quantitative, a statistical comparison was made  
18 between Group 1 and Group 2 for IgG1, IgG2 and IgG levels. The animals belonging to  
19 Group 2 exhibited significantly higher levels of IgG1 ( $p < 0.05$ ), IgG2 ( $p < 0.05$ ) and IgG  
20 ( $p < 0.05$ ) than Group 1.

21

#### 22 3.2. Experimental mucosal infection with a virulent Ba.1 strain of CpHV-1 results in 23 dominant IgG2 responses.

1 A study was then conducted to determine whether infecting the animals by different  
2 mucosal routes or sampling the sera at different time points (post-primary infection) could  
3 result in a different distribution of IgG subclasses. Four goats were experimentally  
4 infected, two by the intravaginal (Figs. 3-5A) and two by the intranasal (Figs. 3-5B) route,  
5 and the kinetics of appearance of total IgG and IgG subclasses in the serum was measured  
6 at different time points post-infection. CpHV-1-specific IgG were detected two weeks  
7 post-infection in all animals and they continued to increase during the following two to four  
8 weeks and reached a maximum level five to eight weeks post-infection (Fig. 3). A parallel  
9 analysis was made for CpHV-1-specific IgG subclasses (Fig. 4) and it was observed that  
10 both IgG1 and IgG2 were detected in comparable amounts two weeks post-infection and  
11 continued to show a similar increase for four weeks post-infection. However, at later time  
12 points, while IgG1 continued to be detected essentially at the same level, IgG2 levels rose  
13 significantly, becoming the dominant subclass in convalescent sera (i.e., five to eight  
14 weeks post-infection). Thus, following primary infection, the final distribution of IgG  
15 subclasses was not influenced by the route of infection; on the other hand, sampling at  
16 different time points determined a different profile of IgG subclasses.

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### 18 **3.3. Contribution of the IgG1 subclass to SN activity**

19 To study the role of the IgG subclasses to CpHV-1 immunity, the SN endpoint titers were  
20 determined in both naturally and experimentally infected animals. An initial comparison  
21 was made between the SN titers obtained with sera from Group 1 and Group 2 of naturally  
22 infected goats. Although there was a slight increase in Group 2 (mean SN titer  $87 \pm 64$  for  
23 Group 2 and  $54 \pm 48$  for Group 1) the differences between Group 1 and 2 were not

1 statistically different. However, with the limited number of animals in Group 2 (n=7) and  
2 the high variability of SN titers in Group 1 (along with variable IgG2 and IgG levels) it was  
3 not possible to obtain a proper statistical comparison. Therefore, among the animals of  
4 Group 1, the few animals exhibiting the same levels of IgG2 as Group 2 but possessing a  
5 significantly lower amount of IgG1 than Group 2 (this subgroup was referred to as  
6 Subgroup 1) were selected and employed for a direct comparison with Group 2 (Table I).  
7 A direct statistical comparison was thus possible since the role of IgG1 in SN could be  
8 evaluated with no interference by IgG2. Table I shows that SN titers and IgG1 levels in  
9 Subgroup 1 were lower than those of Group 2. Finally, the kinetics of the SN endpoint  
10 titers was also determined by using sera from the four experimentally infected goats. Figure  
11 5 shows that, while high SN titers were detected two weeks post infection, they remained  
12 relatively stable in the following five to eight weeks post-infection i.e., at the time when the  
13 IgG2 subclass increased significantly (Fig. 4).

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#### 1 4. DISCUSSION

2 The paucity of data on caprine IgG subclasses, prompted us to develop CpHV-1-specific  
3 ELISAs to measure the distribution of IgG1 and IgG2 in CpHV-1 infected goats. Here we  
4 show that natural and experimental infection resulted in dominant IgG2 responses with  
5 minor production of IgG1 antibodies.

6 The distribution of antigen-specific IgG subclasses can mirror the secretory activity of Th1-  
7 and Th2-type lymphocytes in both humans and animals (Mosmann et al., 1986; 1989;  
8 Finkelman et al., 1990; Parronchi et al., 1991; Stavnezer, 1996; Liblau et al., 1995; Staats et  
9 al., 1994; Marinaro et al., 1998). Thus, in a Th1 dominant setting, the production of IFN-  
10 gamma may promote IgG1 and IgG3 responses in humans (Gregorek et al., 2000; Hussain  
11 et al., 1999; Pène et al., 2004; Widhe et al., 1998), IgG2a in mice (Snapper and Paul, 1987)  
12 and IgG2 in cattle (Estes et al., 1994). On the other hand, Th2-type lymphocytes, secreting  
13 IL-4, support the production of IgG4 in humans (Couper et al., 1998; Hillman et al., 2004;  
14 Ishizaka et al., 1995; Lundgren et al., 1989) and IgG1 in mice and cattle (Snapper et al.,  
15 1988; Estes et al., 1995). Since immune responses in goats resembles those observed in  
16 cattle (Butler, 1983; 1998), the prevalence of CpHV-1-specific IgG2 antibodies reported  
17 here could probably reflect the production of IFN-gamma during herpesvirus infection.

18 The dominance of IgG2 over IgG1 observed in the majority of infected goats is consistent  
19 with data reported with other species infected by herpesviruses. In fact, humoral responses  
20 to HSV in humans are prevalently sustained by IgG1 and IgG3 (Gilljam et al., 1985;  
21 Mathiesen et al., 1988; Sundqvist et al., 1984) and mice produce high levels of IgG2a  
22 antibodies in response to HSV/viral infection (Coutelier et al., 1987; Ishizaka et al., 1995;  
23 McKendall and Woo, 1988). The distribution of antigen-specific IgG subclasses is also

1 informative of the immune defenses mounted by the host (Butler, 1998; Finkelman et al.,  
2 1990; Micusan and Borduas, 1977; Stavnezer, 1996). In this regard, goat IgG2, as well as  
3 human IgG1/IgG3 and mouse IgG2a, bind Fc receptors on macrophages and PMN  
4 (Anderson and Abraham, 1980; Finkelman et al., 1990; Micusan and Borduas, 1977) and  
5 can act as opsonin or promote ADCC with both mechanisms contributing to antibody-  
6 mediated herpesvirus clearance (Butler, 1998; Ferenczy, 2007; Ishizaka et al., 1995; Kohl,  
7 1991; Mathiesen et al., 1988; Micusan and Borduas, 1977). Thus, it could be speculated  
8 that regardless of the species under investigation, the distribution of IgG subclasses  
9 following primary herpesvirus infection is the result of the dominant role played by IFN-  
10 gamma which drives the synthesis of the most appropriate IgG subclass.

11 It should be underlined that IgG4 are also produced in response to primary herpesvirus  
12 infection in humans although HSV-seropositive humans generally exhibit low IgG4 levels  
13 which is in agreement with the low IgG1 levels observed in both naturally infected goats  
14 (Group 1) and experimentally infected goats. Nonetheless, the IgG4 subclass is increased  
15 in patients with nonprimary or recurrent HSV infections (Coleman et al., 1985; Hashido  
16 and Kawana, 1997; Ljungman et al., 1988) or after massive antigenic stimulation (Aalberse  
17 et al., 1983). Thus, either recurrent or nonprimary infections could have contributed to the  
18 high levels of IgG1 (and IgG) found in the minor group of naturally infected goats (Group  
19 2). It also remains to be investigated whether the IgG2 responses can be boosted by a  
20 subsequent CpHV-1 infection since Group 2 exhibited higher IgG2 responses as well.

21 It is remarkable that the SN activity was mainly associated to the IgG1 subclass. Indeed, in  
22 naturally infected goats the SN titer decreased with decreasing IgG1 levels. In addition, in  
23 experimentally infected goats, the comparison of the kinetics of SN with that of IgG

1 subclasses suggested that both subclasses could account for the observed SN activity in the  
2 first two weeks post infection; however, since the IgG2 levels continued to increase five to  
3 eight weeks post infection without causing a parallel increase in the SN endpoint titers, we  
4 speculate that IgG2 may not be directed to neutralizing epitopes. It would be necessary to  
5 purify IgG1 or IgG2 from immune sera in order to study, in vitro and in vivo, the  
6 neutralizing ability of each purified IgG subclass and their specificities.

7 Our data would help to design effective vaccines since vaccination strategies (which are  
8 aimed at preventing infection) should take into account the fact that IgG1 responses could  
9 be more relevant for CpHV-1 neutralization and, therefore, adjuvants and delivery systems  
10 able to potentiate IgG1 (and IgG2) in goats must be preferred (Simms et al., 2002). In  
11 agreement with this, our previous reports have shown that reactivation of infection in  
12 naturally infected goats is more frequent in animals with low SN titers (Tempesta et al.,  
13 1998) and that high SN titers are associated to the production of IgG specific to gB, gC, and  
14 gD, glycoproteins which are involved in viral binding and fusion/penetration (Tempesta et  
15 al., 2005). Finally, IgG1 is the major antibody present in the colostrum and milk of bovines  
16 and goats (Butler, 1983; 1998; Micusan and Borduas, 1976; 1977), thus appropriate  
17 vaccination of adults goats would confer better passive immunity to newborns where  
18 CpHV-1 infection is often lethal.

19 The findings reported here have dual implications. They help dissect the caprine immune  
20 responses to herpesvirus but they are also instrumental to design preventative or therapeutic  
21 tools to be used in goats as well as in humans. In this context, the CpHV-1/goat is an  
22 excellent model to study HHV-2 infection in humans. We have now shown that the profile  
23 of the IgG subclasses in CpHV-1 infected goats mimicks that seen in HHV-2 infected

1 humans. Although mice have been largely used to study HHV-2 infection they may not be  
2 an ideal model (Ferenczy, 2007) while goats may offer selective advantages over rodents  
3 (Hein and Griebel, 2003; Hugo Johansson et al., 1985); however, major efforts have to be  
4 made to overcome the lack of species specific reagents and the absence of detailed  
5 information on the immune system of this veterinary species. The development of assays to  
6 study CpHV-1-specific cellular and humoral immune responses in goats may significantly  
7 impact the field of CpHV-1/HHV-2 research. This is therefore the focus of our current  
8 investigation.

9

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1 **Legends to Figures**

2

3 **Figure 1**

4 CpHV-1-specific serum IgG in naturally infected goats. Sixty-three goat sera were screened  
5 by using a CpHV-1-specific IgG ELISA. There were 43 seropositive animals which were  
6 then used to analyze the CpHV-1-specific IgG subclasses while the 20 seronegative goats  
7 were not included in the subsequent study. Reported data are mean + SD.

8

9 **Figure 2**

10 Distribution of CpHV-1-specific serum IgG1 and IgG2 in the 43 naturally infected goats.  
11 IgG subclass levels were determined by using CpHV-1-specific IgG1 and IgG2 ELISAs.  
12 The distribution of IgG subclasses in the 43 seropositive goats segregated into two different  
13 profiles. A major group of animals (named Group 1 and consisting of 36 out of 43 goats)  
14 exhibited significantly higher IgG2 levels than IgG1 (Fig. 2A;  $p < 0.05$  by the Student's t  
15 test). A minor group of seropositive goats (named Group 2 and consisting of 7 out of 43  
16 goats ) exhibited equal levels of IgG1 and IgG2 (B).

17

18 **Figure 3**

19 Kinetics of CpHV-1-specific serum IgG responses following experimental CpHV-1  
20 infection. Four goats were experimentally infected with a virulent CpHV-1 Ba.1 strain; two  
21 goats were infected by the intravaginal route (A) and two goats were infected by the  
22 intranasal route (B). At the indicated time points post-infection, sera were taken and CpHV-  
23 1-specific IgG levels were measured by ELISA. Reported data are mean + SD.

1

2 **Figure 4**

3 Kinetics of CpHV-1-specific serum IgG1 and IgG2 responses following experimental  
4 CpHV-1 infection. Four goats were experimentally infected with a virulent CpHV-1 Ba.1  
5 strain; two goats were infected by the intravaginal route (A) and two goats were infected by  
6 the intranasal route (B). At the indicated time points post-infection, sera were taken and  
7 CpHV-1-specific IgG1 and IgG2 levels were measured by ELISA. Reported data are mean  
8 + SD.

9

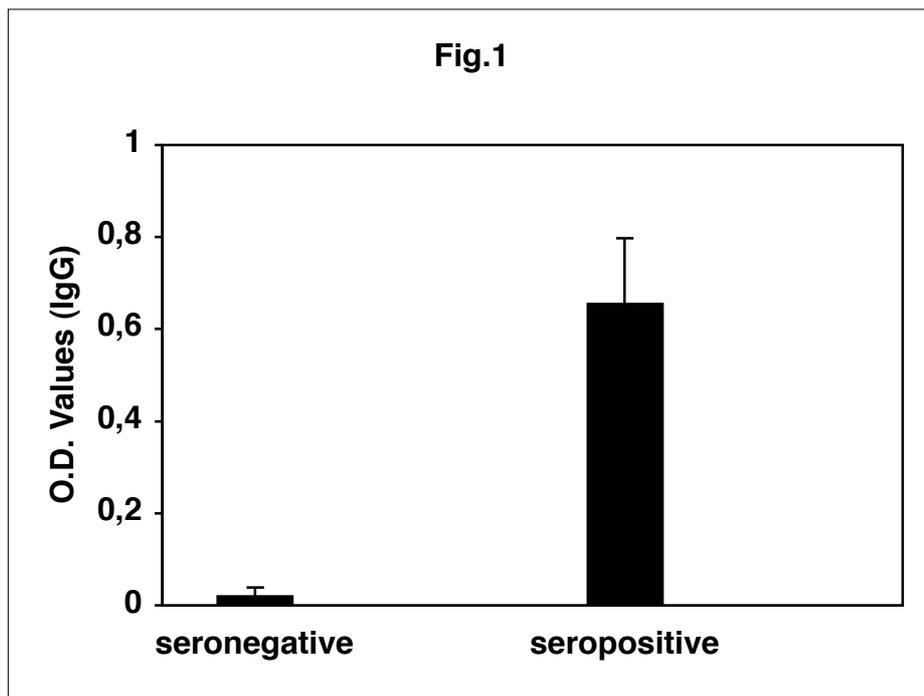
10 **Figure 5**

11 Kinetics of SN endpoint titers following experimental CpHV-1 infection. Four goats were  
12 experimentally infected with a virulent CpHV-1 Ba.1 strain; two goats were infected by the  
13 intravaginal route (A) and two goats were infected by the intranasal route (B). At the  
14 indicated time points post-infection, sera were taken and SN assays were performed. The SN  
15 endpoint titers were determined as the highest serum dilution neutralizing the cytopathic  
16 effect of the virus in vitro. Reported data are mean + SD.

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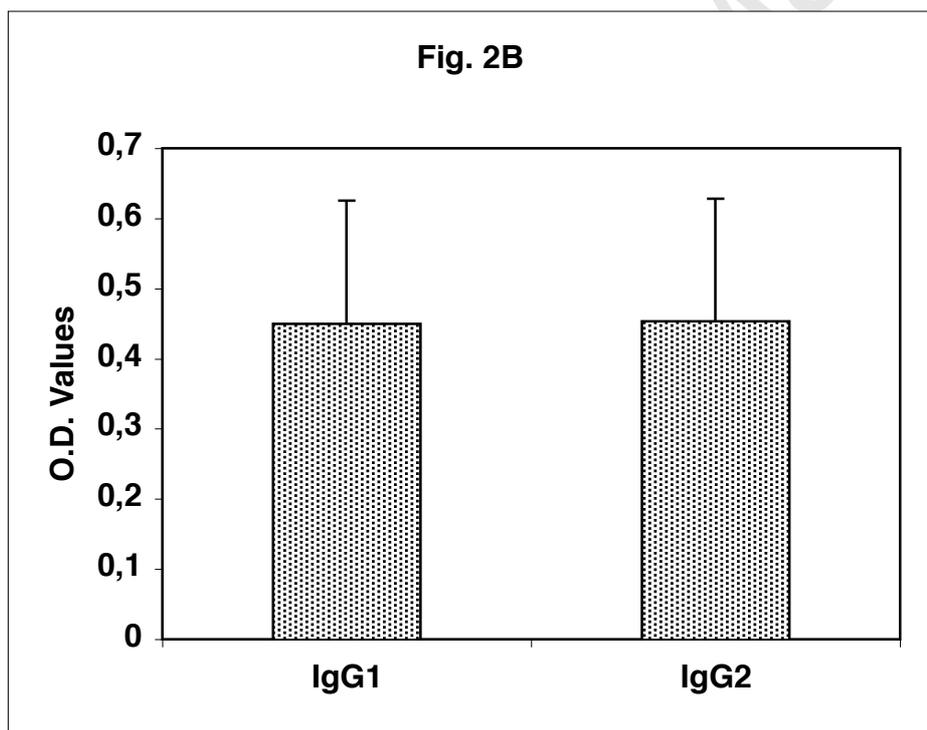
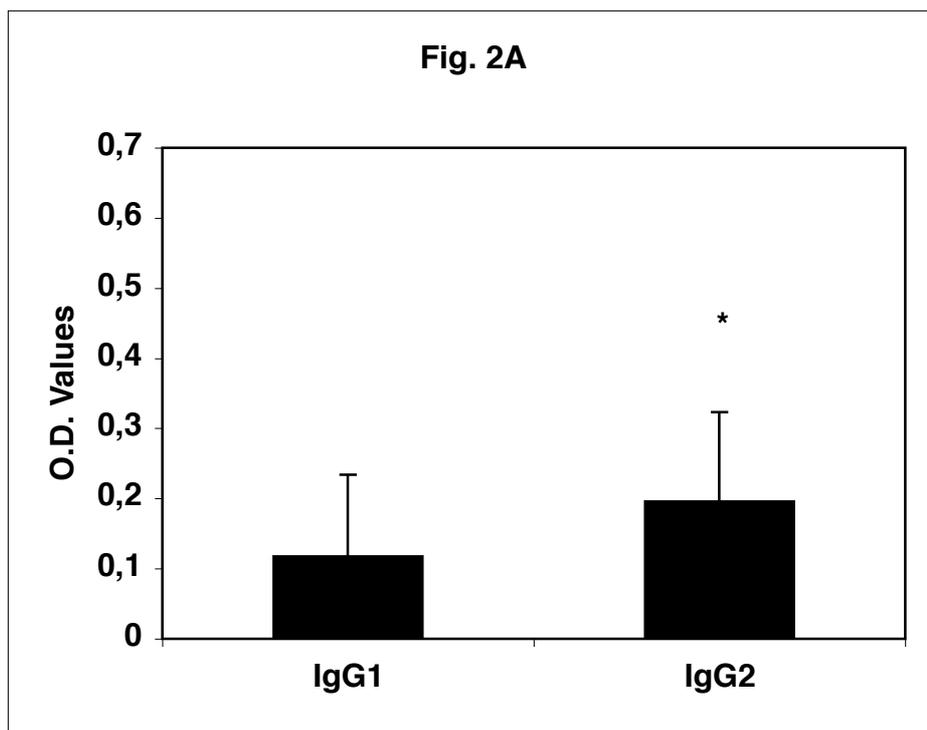


Fig. 3A

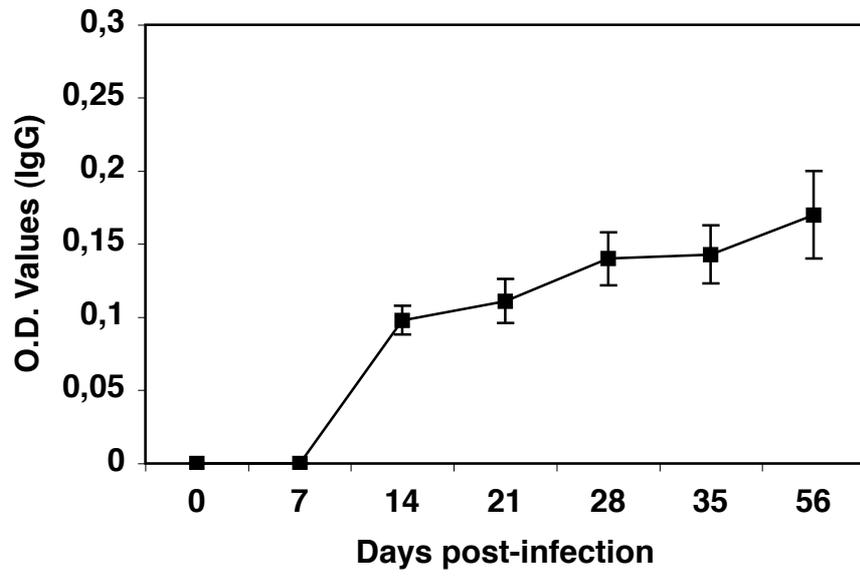


Fig. 3B

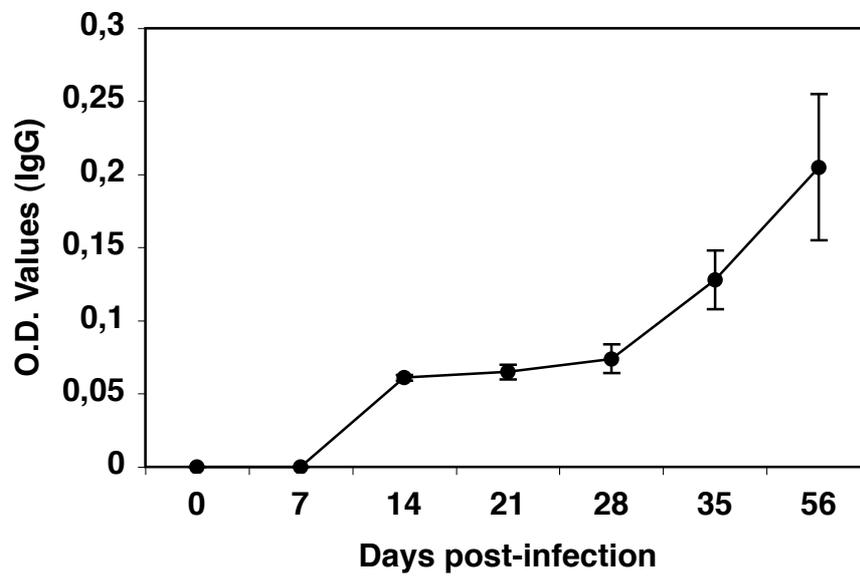


Fig. 4A

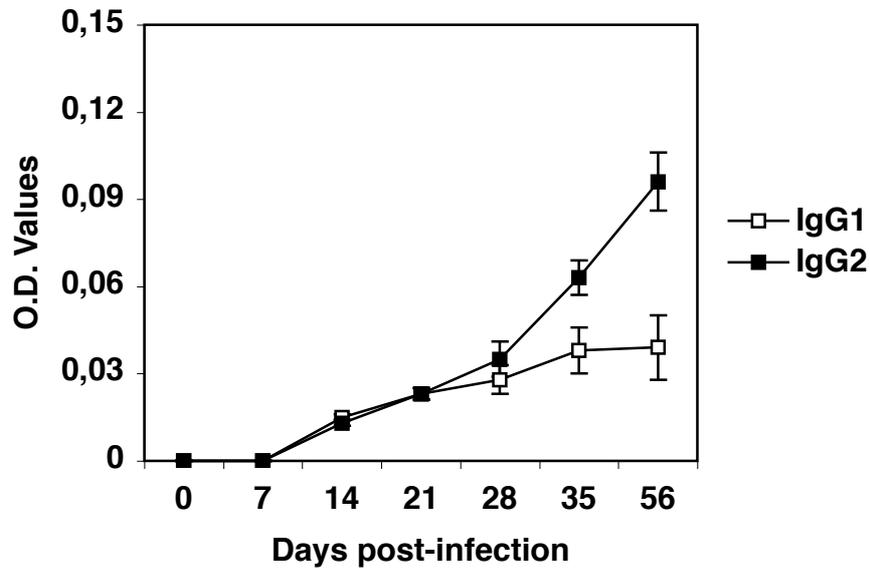


Fig. 4B

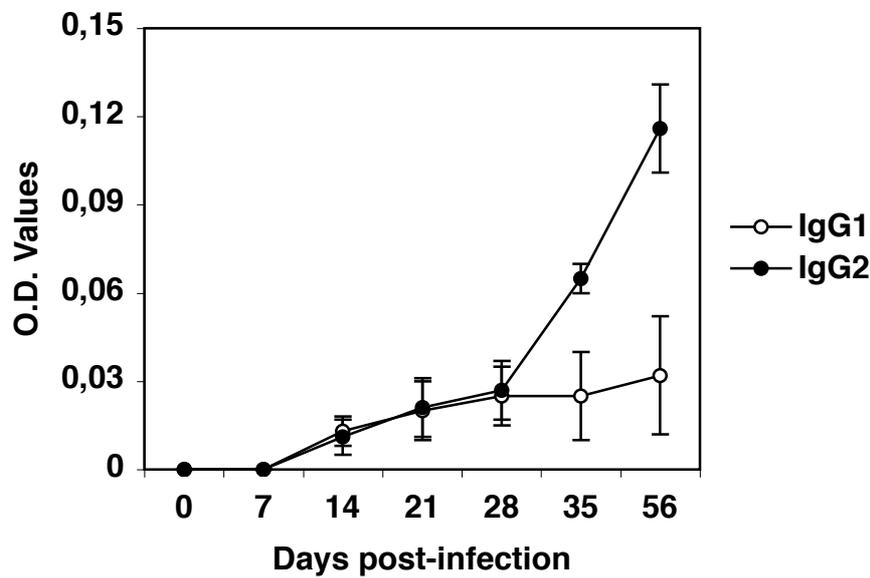


Fig. 5A

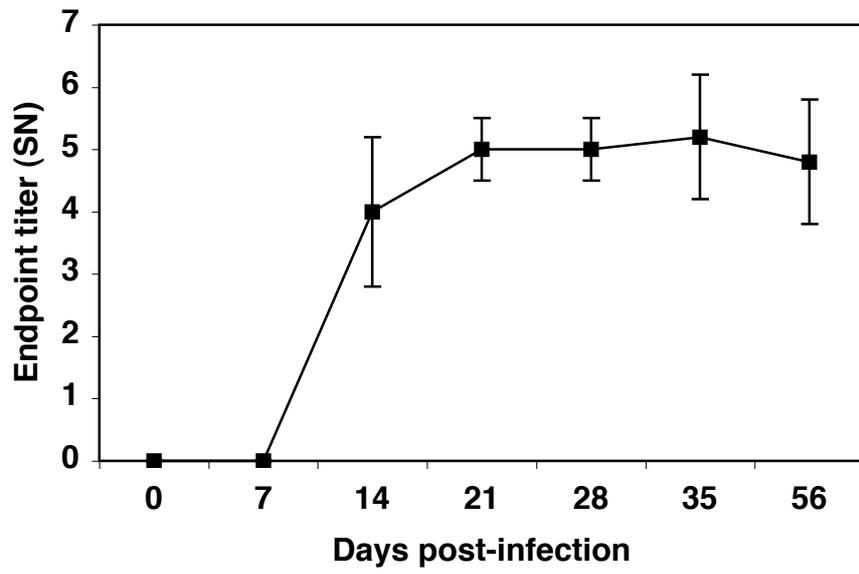
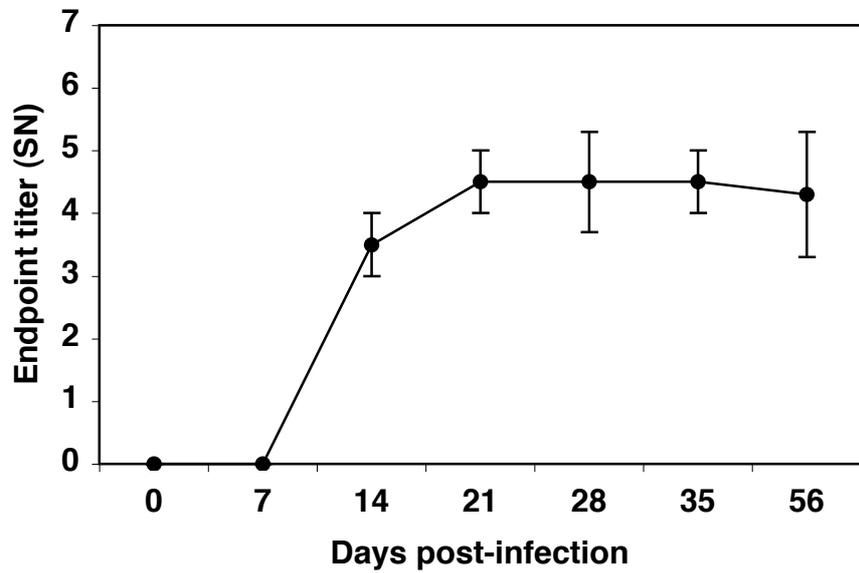


Fig. 5B



1 **Table I** CpHV-1-specific IgG1 are mainly associated to the SN activity in  
 2 naturally infected goats

	SN titer +/- SD	IgG1 +/- SD <sup>a</sup>	IgG2 +/- SD <sup>a</sup>
3			
4			
5			
6			
7			
8			
9	<b>SubGroup 1<sup>#</sup></b>	29 +/- 16	0.216 +/- 0.136
10			
11			
12	<b>Group 2</b>	87 +/- 64 *	0.450 +/- 0.175 *
13			
14			

15 <sup>a</sup> ELISA O.D. Value

16

17 <sup>#</sup> Among the 36 goats of Group 1, a subgroup of 6 goats was selected according to the  
 18 following criteria: IgG2 levels were not significantly different from Group 2; IgG1  
 19 levels significantly lower than those of Group 2. The SN titers of the Subgroup 1 and  
 20 Group 2 were then statistically compared.

21

22 \*p value < 0.05 Group 2 vs SubGroup 1 by the ANOVA test

23