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## **Viral infections in free-living populations of the European wildcat**

Leutenegger, Christian M; Hofmann-Lehmann, Regina; Riols, C; Liberek, M; Worel, G; Lups, P; Fehr, D; Hartmann, M; Weilenmann, P; Lutz, Hans

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## VIRAL INFECTIONS IN FREE-LIVING POPULATIONS OF THE EUROPEAN WILDCAT

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**ABSTRACT:** While the importance of viral infections is well studied in domestic cats, only limited information is available on their occurrence and prevalence in the European wildcat (*Felis silvestris silvestris*). The aim of this study was to determine the prevalence of antibodies to feline coronavirus (FCoV), calicivirus (FCV), herpesvirus (FHV), parvovirus (FPV), immunodeficiency virus (FIV), leukemia virus (FeLV), and FeLV antigenemia in 51 European wildcat sera. Samples were collected between 1996 and 1997 from wildcat populations in France, Switzerland, and Germany. Antibodies to FCoV were detected in two cats (4%) and FCoV RNA was detected in feces of one of these two cats. Antibodies to FCV, FHV and FPV were found at relatively low frequencies of 16%, 4%, and 2%, respectively. Antibodies to FIV were not detected. Although antigen and antibodies to FeLV were detected in 49% and 75%, respectively, no evidence of FeLV-associated pathology was found. From the low prevalence of FCoV, FCV, FHV and FPV infections and from the fact that the European wildcats live solitarily, it was concluded that these viral infections do not spread readily within a population. Therefore, it may be assumed that release into the wild of European wildcats bred in captivity would not bring about a high risk of introducing of these viral infections to the free-ranging wildcats. As an exception, wildcats should be tested for absence of FIV infection before release if they were at risk to acquire this infection from domestic cats.

**Key words:** European wildcat, feline calicivirus, feline coronavirus, feline herpesvirus, feline immunodeficiency virus, feline infectious peritonitis, feline leukemia virus, feline parvovirus, *Felis silvestris silvestris*, serosurvey.

### INTRODUCTION

The European wildcat (*Felis silvestris silvestris*) is a threatened species in Europe and according to the CITES agreement (Goldsmith, 1978) capture and trade of animals and parts thereof are highly restricted. Survival of European wildcat populations has been aided by release of wildcats bred in captivity into the wild (Büttner, 1994), but there are few published reports about the prevalence and possible pathogenic aspects of viral infections in these populations (Artois and Remond, 1994; Boid et al., 1991; McOrist et al., 1991).

Several viruses of domestic cats are known to exist also in nondomestic felid populations: feline coronavirus (FCoV), fe-

line calicivirus (FCV), feline viral rhinotracheitis virus (feline herpesvirus, FHV), feline panleukopenia virus (feline parvovirus, FPV), and feline immunodeficiency virus (FIV) (Paul-Murphy et al., 1994; Spencer, 1991; Hofmann-Lehmann et al., 1996; Olmsted et al., 1992; Lutz et al., 1992). Only feline leukemia virus (FeLV) is not regularly found in free-ranging wild felids. Some of the reports of FeLV infection in wild felids may be explained by close contacts with domestic cats (Meric, 1984; Jessup et al., 1993).

In domestic cats, infection with feline coronavirus (FCoV) occurs worldwide and eventually may lead to a lethal disease called feline infectious peritonitis (FIP). Antibodies to FCoV are present in 80 to 90% of the cats in catteries and in 10 to

50% of single-cat households (Addie and Jarrett, 1992; Sparkes et al., 1992; Herrewegh et al., 1995; Pedersen, 1976; Fehr et al., 1997). Up to 5 to 10% of seropositive cats die of FIP (Addie and Jarrett, 1992; Pedersen, 1976; Fehr et al., 1997) and it is believed that factors such as susceptibility, age, stress, and virus load influence the outcome of an infection with FCoV. Infection with FCoV leading to FIP has also been reported to have devastating effects in some wild felids kept in captivity; FIP cases have been reported mainly in cheetahs but also in African lions (*Panthera leo*), leopards (*Panthera pardus*), mountain lions (*Felis concolor*), lynx (*Lynx lynx*), caracal (*Felis caracal*), and smaller wildcats (Colby and Low, 1970; Evermann et al., 1983; Quensberry, 1984; Van Rensburg and Silkstone, 1984). FCoV has recently been reported in free-ranging Scottish European wildcats (*Felis silvestris silvestris*) (Daniels et al., 1999), and outbreaks have been described in two closed colonies of European wildcats (Lutz et al., 1996; Watt et al., 1993).

Feline calicivirus (FCV) and feline herpesvirus (FHV) are agents causing mainly upper respiratory tract diseases. FCV in most instances is responsible for stomatitis, gingivitis, and glossitis. Clinical signs of rhinotracheitis have been described in wildcats from Scotland (McOrist et al., 1991) and serological evidence of FCV infections was detected in wildcats from eastern France (Artois and Remond, 1994).

Feline parvovirus (FPV) is a highly contagious virus prevalent in most members of the family Felidae. It induces an acute disease characterized by leukopenia, fever, depression, dehydration, diarrhea, and death in domestic cats. A majority of the free-ranging mountain lions in California (93%; Paul-Murphy et al., 1994), the African lions in Kruger National Park (84%; Spencer, 1991) as well as the lions in the Serengeti and Ngorongoro Crater had antibodies to FPV (Hofmann-Lehmann et al., 1996). Antibodies to FPV occasionally

could be found in European wildcats but due to low numbers of examined samples, a reliable prevalence estimate could not be determined (Artois and Remond, 1994).

Feline immunodeficiency virus (FIV) infection is associated with a progressive immunosuppression and leads to opportunistic infections in domestic cats. Viruses closely related to the FIV of domestic cats occur also in nondomestic felid species: members of at least 18 of the 37 species in the family Felidae carry an FIV-related virus as has been shown by the presence of antibodies in their sera crossreacting with FIV antigen (Hofmann-Lehmann et al., 1996; Olmsted et al., 1992; Brown et al., 1994; Carpenter and O'Brien, 1995; Lutz et al., 1992). Currently, FIV is not considered to be present in the European wildcat (Artois and Remond, 1994; McOrist et al., 1991; McOrist, 1992).

Feline leukemia virus (FeLV), an exogenous retrovirus that is transmitted horizontally and vertically among domestic cats, causes neoplastic diseases including lymphosarcoma and leukemia, aplastic anemia and immunosuppression in the domestic cat (Jarrett et al., 1964). This virus has consistently been found in the European wildcat. It was detected at a prevalence of 9% in a UK population (Boid et al., 1991; McOrist, 1992) and at 40% in the eastern France population (Artois and Remond, 1994). Reports on FeLV in exotic felids have been based on positive antigen-tests in occasionally captive animals (Boid et al., 1991; Meric, 1984; Citino, 1986), the isolation of the virus from a leopard cell line (Rasheed and Gardner, 1981) and from an European wildcat in Scotland (Boid et al., 1991). Furthermore, Jessup et al. (1993) described a FeLV-positive cougar which might have acquired the infection from a FeLV positive domestic cat, based on being found in a city.

It was the goal of the present study to determine the prevalence and the possible biological importance of viral infections in European wildcat populations in central eastern France, Germany, and Switzerland.

## MATERIALS AND METHODS

### Population structure

The wildcats in France and Switzerland are part of autochthonous populations, whereas the animals in Germany are captive bred and later released into the wild through the reintroduction program in Bavaria. This species is covered by the CITES agreement as it is considered a threatened species. However, no information is available on the number of animals living in the different habitats and the true density of the population is unknown. Cats were defined as wildcats by phenotypical assessment (i.e., size of the skull) and also according to anatomical criterias (i.e., length of intestine; Schauenberg, 1977; Piechocki, 1986).

### Animals and sampling of blood and feces

Blood (heart clot) and fecal samples were obtained from 9 wildcats found dead in the Swiss Jura formation (47°00'N, 6°95'W) from 1996 to 1997. Bodies were immediately frozen after collection. Blood and fecal samples were collected after thawing at necropsy, and the samples were immediately frozen, transported on dry ice and stored at -80 C until tests were performed.

In addition, blood samples were collected from 34 wildcats in France which had been hit by car between March 1995 and October 1996. Collected animals were frozen at -30 C and stored for one to several weeks. Blood and fecal samples were collected as described above. After thawing, the blood samples were centrifuged at  $15,000 \times g$  for 20 min at 4 C to remove cell debris, and used for serological assays.

Eight serum samples were obtained from anesthetized subadult wildcats bred in captivity, which were to be released by the German release program in Wiesenfelden near Straubing (Bavaria, Germany, 48°53'N, 12°34'W).

### Serology

Antibodies to FCoV, FCV, FHV, and FPV were detected by immunofluorescence assay (IFA). The substrate for the FCoV assay was TGEV in crandell feline kidney cells (CrFK) cells (Lutz et al., 1985; Osterhaus et al., 1977), for calicivirus CrFK infected with FCV (Swiss strain, kindly provided by Veterinaria, Zurich, Switzerland), for the FHV assay CrFK infected with FHV (Swiss strain, kindly provided by Veterinaria, Zurich, Switzerland; Hofmann-Lehmann et al., 1996), for the FPV assay CrFK infected with a Swiss strain of FPV (Hofmann-Lehmann et al., 1996). All IFAs were tested at dilutions of 1:25, 1:100, 1:400, 1:1600. The con-

jugate was goat anti-cat FITC (KPL, Gaithersburg, Maryland, USA).

Antibodies to the transmembrane (TM) protein of FIV were detected by enzyme-linked immunosorbent assay (ELISA) as described (Calzolari et al., 1995) and by Western blot (Lutz et al., 1988).

Antibodies to FeLV gp70 were detected by ELISA described elsewhere (Lutz et al., 1980).

### Viral detection

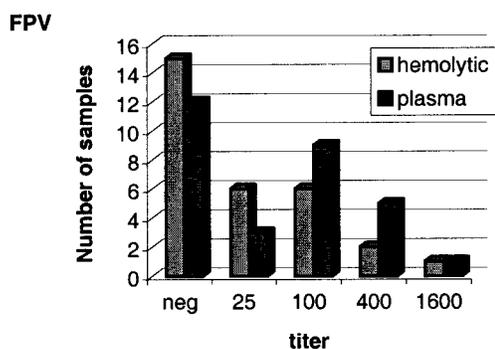
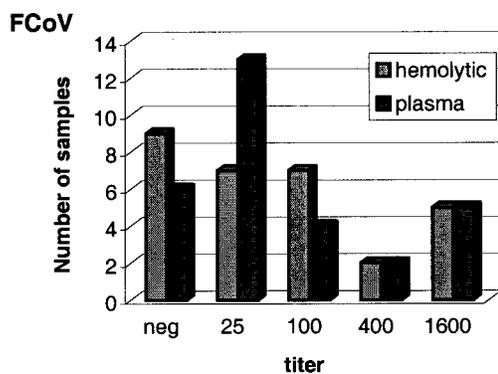
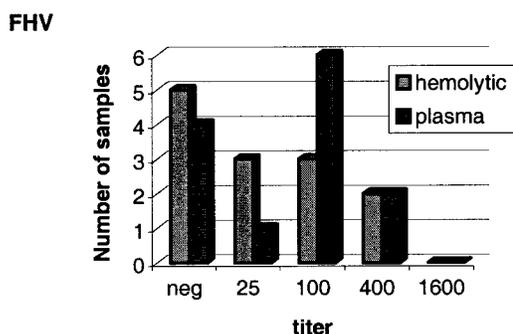
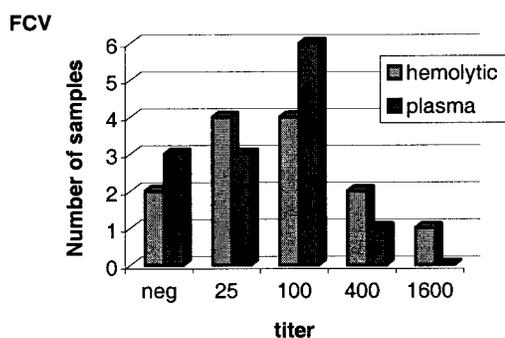
A nested RT-PCR was carried out as described (Fehr et al., 1996) amplifying a highly conserved 3'-untranslated region (3'-UTR) of the FCoV genome.

The serum samples were tested for presence of FeLV p27 by a double-antibody sandwich ELISA based on monoclonal antibodies (Lutz et al., 1983).

### Sensitivity tests

Due to the freezing process, serum samples collected from dead animals were hemolyzed. In order to determine whether and to what degree the hemolysis affected the serological assays, 30 whole blood domestic cat samples were tested. The initial serological tests were done using EDTA plasma. In order to obtain hemolytic samples, aliquots of the same whole blood samples were subjected to one freeze-thaw cycle, centrifuged, and serologic tests for FCoV, FCV, FHV, and FPV antibodies were repeated.

In order to determine whether hemolysis was responsible for positive FeLV p27 ELISA results, p27 antigen was immune-complexed, precipitated with polyethylene glycol (PEG 6000, Fluka, Buchs, Switzerland) and tested in Western blot. The experiments were carried out using the following volumes and reagents: to 100  $\mu$ l of the hemolytic wildcat blood and of a FeLV positive domestic cat blood serving as a positive control, 20  $\mu$ l of a polyclonal goat serum specific for FeLV p27 (serum no. 78-S; National Cancer Institute, Bethesda, Maryland, USA) were added. Forty  $\mu$ l of 10% polyethylene glycol buffer (5 g polyethylene glycol 6000 in a total of 50 ml phosphate buffered saline) were added to 100  $\mu$ l each of the hemolytic blood samples of these cats. The anti p27 serum and the hemolytic blood samples were incubated in a rotatory shaker for three hours at 37 C; incubation was continued at 4 C overnight. Tubes were centrifuged ( $10,000 \times g$  at 4 C) and the resulting pellets were washed three times in 1.5 ml 2.5% PEG wash solution (10 mM Tris-HCl, pH 7.2, 25 mM sodium borate, 140 mM NaCl, 10 mM Na-EDTA, 2.5% PEG 6000) and centrifuged as above. The final pel-



lets were dried in a nitrogen stream and solubilized in 50  $\mu$ l of 6 M urea. After addition of 5  $\mu$ l of 4  $\times$  SDS sample buffer, the samples were heated for 5 min at 95 C and loaded on a 10% polyacrylamide gel. Proteins were blotted to a nitrocellulose sheet and incubated with a mixture of monoclonal antibodies to p27 conjugated to peroxidase (10  $\mu$ l/ml) or a polyclonal anti FeLV p27 serum (as used above) conjugated to peroxidase. Detection of an antigenic band of 27,000 da on the Western blot strip by monoclonal antibodies to p27 was considered proof for the presence of FeLV p27 in the hemolyzed samples.

## RESULTS

### Sensitivity tests

In the FPV IFA, 15 of 30 (50%) samples tested positive with hemolytic samples, whereas 18 of 30 (60%) samples tested positive with nonhemolytic plasma samples. For the FPV IFA, there was a 10% loss of sensitivity. Similar results were obtained for FCoV (10% loss of sensitivity), FCV (8% gain of sensitivity), and FHV (7% loss of sensitivity). For details see Figure 1. From these experiments it was concluded that the use of hemolyzed blood samples would result in slightly decreased numbers of positive tested samples, in the case of FCV with unchanged or even slightly increased numbers of samples tested positive for FCV compared with the use of non-hemolytic plasma.

### Performance of the FPV IFA assay and of FeLV p27 ELISA

Seventeen plasma samples from domestic cats vaccinated twice against FPV prior to the IFA were tested for antibodies to parvovirus as described above. One of them was seronegative, five showed a titer of 80, one had a titer of 160, four a titer of 320, five a titer of 1,500, and one a titer of 2,000.

The specificity of the FeLV p27 antigen

←

FIGURE 1. Effect of hemolysis in serum samples from the European wildcat on the performance of the FCV, FHV, FPV and FCoV (see text for abbreviation) immunofluorescence assays.

ELISA was confirmed by precipitating p27 antigen in four of four hemolytic blood samples of the wildcats that were positive for p27 by ELISA. At the same time, two serum samples from FeLV negative spf cats were clearly negative for p27 by Western blot analysis of the PEG precipitated immune complexes.

#### Prevalence of antibodies to FCoV, FCV, FIV, FHV, FeLV and FPV

The prevalences of antibodies to FCoV, FCV, FIV, FHV, FeLV and FPV were as follows (Table 1): two of the 51 cats (4%) tested positive for FCoV. Both FCoV antibody positive cats were wildcats without evident signs of preexisting disease. Eight of the 51 (16%) tested samples showed antibodies to FCV: five cats of the French population, two cats from the wildcats from Germany, and one cat from the Swiss population. No antibodies to FIV could be detected in any of the 51 tested serum samples. Antibodies against FHV were found in two wildcats (4%). Twenty-five of the 51 samples (49%) tested positive for antibodies to FeLV. One cat (2%) showed antibodies to FPV.

#### Detection of FCoV RNA and FeLV antigen

Fecal samples from all cats of the Swiss and 26 cats of the French wildcat population were analyzed by RT-PCR to detect FCoV RNA. The animal from the French population with a titer of 100 by FCoV IFA was also positive by RT-PCR. All other cats were negative in their feces (98%). FeLV p27 antigen was detected in 77% of all serum samples.

#### DISCUSSION

Some of the samples used in this study originated from cats which had been exposed to decay for an unknown duration. In view of the fact that antibodies are known to be quite resistant to proteolytic degradation (Bazin et al., 1994), we speculate that no loss in titers have to be expected within 48 hr after death of the French wildcats which was considered to

TABLE 1. Prevalence of antibodies to feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), feline calicivirus (FCV), feline herpesvirus (FHV), feline parvovirus (FPV) in serum and prevalence of FeLV antigenemia and FCoV viral RNA in feces of the European wildcat in central Europe.

	Sex	Samples	FeLV		FIV		FCoV		FCV <sup>a</sup> ab <sup>a</sup>	FHV <sup>a</sup> ab <sup>a</sup>	FPV <sup>a</sup> ab <sup>a</sup>
			p27	ab <sup>b</sup>	ab <sup>b</sup>	WB	ab <sup>a</sup>	viral RNA			
France	Male	19	14 (74%)	5 (45%)	0 (0%)	0 (0%)	1 (5%)	1 (5%)	2 (11%)	1 (5%)	0 (0%)
	Female	15	12 (80%)	6 (55%)	0 (0%)	0 (0%)	1 (7%)	0 (0%)	3 (20%)	0 (0%)	0 (0%)
Switzerland	Total	34	26 (76%)	11 (32%)	0 (0%)	0 (0%)	2 (6%)	1 (5%) <sup>c</sup>	5 (15%)	1 (3%)	0 (0%)
	Male	7	6 (86%)	6 (86%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Germany <sup>d</sup>	Female	2	2 (100%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)
	Total	9	8 (89%)	8 (89%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (13%)	1 (13%)	0 (0%)
All areas	Female	8	4 (50%)	6 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (25%)	0 (0%)	1 (13%)
	Total	51	38 (75%)	25 (74%)	0 (0%)	0 (0%)	2 (4%)	1 (5%) <sup>c</sup>	8 (16%)	2 (4%)	1 (2%)

<sup>a</sup> Tested by IFA.

<sup>b</sup> Tested by ELISA; WB,

<sup>c</sup> n = 22.

<sup>d</sup> No information available about sex.

be the patrol interval of the inspection tours. The situation of cats collected in Switzerland is somewhat different because time between death and recovering cannot be estimated. However, 5 of 9 cats were found during wintertime. The fact that five of nine cats collected in Switzerland had antibodies to FeLV supports our assumption that antibodies were still active. The serological tests of this study had to be done with hemolytic samples. The results showed that hemolysis leads to a decrease of sensitivity of less than one titer-step corresponding to the volume displacement effect caused by the red cells present in the blood samples (hematocrit, 30–40%). In consideration of the above mentioned experiments we may have underestimated the true prevalence of the infections. However, the misjudgment may not exceed 10%.

The FCoV results are in contrast to the situation of the domestic cat, where an antibody prevalence of 50 to 90% can be detected (Pedersen, 1976; Fehr et al., 1997). In contrast to the domestic cat, free-living wildcats are living as a dispersed social system. As contacts between individual wildcats occur mainly in connection with mating (Parent, 1975) and at low frequency, a decreased risk to spread infectious diseases can be postulated. It was shown that in domestic cats, FCoV infection can be controlled if the number of cats living in a confined area is reduced (Addie and Jarrett, 1992). The fact, that FCoV infection is present at low prevalence in the European wildcat is therefore in agreement with the observation made by Addie and Jarrett (1992). Even if asymptomatic wildcats exist as FCoV carriers, as was described for domestic cats (Addie and Jarrett 1992; Pedersen, 1987), they are only able to spread the virus in a limited area and to a low number of other wildcats. The two FIP outbreaks in closed colonies showed that European wildcats kept under captive conditions that imply an unecological crowding are also susceptible to FCoV infection and FIP. This situation is very

similar to that of the domestic cat; FIP occurs mainly in cats living in densely populated groups. Release of FCoV seropositive wildcats into the wild will relieve the animals from the infectious pressure experienced while living in a group in captivity. We speculate that with time the antibody titers and most likely also the FCoV load will decrease to levels below the detection limit. It would be interesting to know whether wildcats in which the FCoV antibodies decrease to an undetectable level are able to completely get rid of FCoV or whether the virus can persist somewhere in the cats' body.

Antibodies to FCV, FHV, and FPV were only rarely detected in the European wildcat populations. This came as a surprise, especially in the case of FPV known to be not only highly contagious but also forms a reservoir of infection in the domestic cat. The efficiency of the FPV IFA to detect antibodies specific for FPV was evaluated by testing plasma samples from cats vaccinated against FPV one year prior to the IFA. These results indicated, that the test reliably detects FPV antibodies in the plasma samples. Furthermore, these tests show that the low prevalence of antibodies to FPV, FCV, and FHV in the European wildcat population is real and not due to insensitive tests.

Lentiviruses related to FIV of domestic cats have been isolated and described from a wide variety of species in the family of the Felidae (Hofmann-Lehmann et al., 1996; Olmsted et al., 1992; Brown et al., 1994; Carpenter and O'Brien, 1995; Lutz et al., 1992). However, it has not yet been demonstrated that FIV-related viruses cause disease in species other than the domestic cat (Brown et al., 1994; Lutz et al., 1992). Similar to investigations made by other groups (Artois and Remond, 1994; McOrist et al., 1991; McOrist, 1992), no evidence for the presence of a European wildcat-related FIV could be found in the present study. It has to be considered, that all these attempts to detect FIV in wildcats were based on the possible ability of an-

tibodies to crossreact with FIV antigens derived from domestic cats. All lentiviruses so far detected in wild felids show immunological crossreactivity with core proteins of FIV of the domestic cat (Barr et al., 1995). Therefore, our results exclude the existence of a wildcat lentivirus closely related to that of the domestic cat but not one more closely related to ovine or equine lentiviruses.

Both free-ranging wildcat populations showed a high prevalence of FeLV p27 antigen and antibodies to FeLV. From the results of the immune-coprecipitation it was concluded, that hemolysis was not responsible for the positive results of the p27 ELISA and that the high prevalence found in our study indeed corresponded to a high prevalence of FeLV infection in the wildcat population. These results are in agreement with other studies reporting a high prevalence of FeLV in different wildcat populations (Boyd et al., 1991; McOrist, 1992; Artois and Remond 1994). The cats of the present study originating from France ( $n = 34$ ) and Switzerland ( $n = 9$ ) were all victims of car accidents and therefore our findings may be biased. Therefore the true prevalence of FeLV may have been overestimated. However, the eight subadult wildcats bred in captivity and prepared to be released into the wild also showed a high prevalence of FeLV antigen and antibodies to FeLV (FeLV p27 antigen: 50%, FeLV antibodies: 75%) in the absence of clinical signs. Currently, it is difficult to estimate the disease potential of FeLV or whether several strains with different pathogenicity are present in wildcats. To what extent FeLV infected wildcats can serve as a reservoir for domestic cats is unknown; however, transmission of FeLV from wild to domestic cats could theoretically occur.

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