

SEROLOGIC SURVEY IN A CHAMOIS POPULATION OF ABRUZZO

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ABSTRACT - As part of the Abruzzo National Park wildlife health management program, serum samples from 62 free-living and captive Abruzzo chamois, *Rupicapra (pyrenaica) ornata*, were tested to estimate antibody presence to some pathogenous agents. The serum, drawn during chemical immobilisations for introductions or population study programs, were tested for: bovine *herpes virus 1* (BHV-1), *parainfluenza virus* (PI3), *pesti virus*, foot and mouth disease virus (FMD) type O-A-C, bovine leukemia virus (BLV), *ovicaprini lentivirus*, *encephalomyocarditis virus* (EMCV), *S-phase hrucellae*, *leptospira interrogans*, *mycobacterium paratuberculosis*, *chlamydia psittaci*, *coxiella burnetii*, *rickettsia mooseri* and *conori* and *toxoplasma gondii*. Twenty-three subjects were found positive to BHV-1 (7/62), *pestivirus* (6/62), EMCV (8/62), *leptospira* (5/62) and *toxoplasma* (4/62). Results did not indicate any active infection but only contact with some pathogenic agents.

Key words: Abruzzo chamois, serologic survey, serum positivity, pathogenic agents.

INTRODUCTION

The Apennine or Abruzzo chamois, fully protected by Italian law, was identified as vulnerable by I.U.C.N. (Thornback, 1980) and listed in Appendix 1 of the 1973 Washington Convention.

At present, the only remaining population of Abruzzo chamois comprises some 600 individuals. These include most of the population living in Abruzzo National Park (Central Italy), two free-living herds recently introduced to Majella and Gran Sasso National Parks and animals in captivity. Further details may be found in Tassi *et al.* (1992). This work is part of a wildlife health management and research program, and reports on some preliminary results obtained in research on the immune status of the Abruzzo chamois population living in Abruzzo National Park.

By analysing serum samples of animals from different areas, we tried to find out if there were some pathogens, common in domestic ruminants, which could be a source of infection for chamois.

MATERIAL AND METHODS

Between July 1990 and November 1993, during marking or introduction operations, 85 blood samples were collected from 62 Abruzzo chamois (31 males, 31 females, 1-16 years of age) anaesthetised with xylazine and ketamine (Locati *et al.*, 1991).

A sample of 30 sera were collected from 27 free-ranging animals captured in Val di Rose, a valley in the heart of the chamois range where density was about 20 individuals/km² (Lovari, 1985). This area is part of the Park integral reserve where domestic livestock are

excluded and visitors are restricted. The main animals living in the chamois area are the brown bear, wolf, red deer, wild boar and various small mammals and birds.

The other sample of 55 sera were collected from 35 chamois kept in enclosures of about 3-4 hectares in area (Wildlife Areas), in Abruzzo and Majella National Parks.

Some individuals were tested twice or more with intervals ranging from 12 days to 2 years. Blood samples were removed from the jugular vein using a 10ml vacuum blood sample tube (Vacutainer). The tubes were centrifuged at 3000 r.p.m. for 15 minutes, within 12 hours to obtain serum, then samples were frozen until examination. The tests used for the detection of antibodies against different viruses considered are reported:

Bovine herpes virus (BHV-1)

Microseroneutralisation (MSN) on serum inactivated at 56°C for 30 minutes, with an initial dilution of 1:2 using 100 TCID₅₀ of a strain of BHV-1 and, as a substrate, a continuous line bovine kidney cell culture (Aubek). The neutralising titre was expressed as the reciprocal of the highest dilution of serum able to completely neutralise 100 TCID₅₀ of virus.

Parainfluenza 3 virus (PI3)

Haemoagglutination inhibition (HI) on serum pre-treated with potassium periodate at 4°C for 18 hours with starting dilution 1:5, using 4 UHA of a strain of bovine PI3 and guinea pig red blood cells at 0.75%. The HI titre was expressed as the reciprocal of the highest serum dilution able to completely neutralise 4 UHA of virus.

Pesti virus

Enzyme-linked immunosorbent assay (ELISA) type blocking on serum prediluted 1:2 and using the pesti virus kit supplied by the Brescia Institute of Zooprophyllaxis. The serum titre was expressed as the reciprocal of the dilution that could provoke a 50% reduction of the DO of reference (control 100%) corresponding to a total absence of competition.

Foot and Mouth disease virus (FMDV) type O-A-C

ELISA type blocking on serum with initial dilution 1:10, using the kit supplied by the Brescia Institute of Zooprophyllaxis. The serum titre was expressed as the reciprocal of the dilution that could provoke a 50% reduction of the DO of reference (control 100%) corresponding to a total absence of competition.

Bovine leukemia virus (BLV)

Agar gel immunodiffusion (ACID), using glycoprotein antigen and positive reference serum from the Perugia Institute of Zooprophyllaxis.

Ovicapriini lentivirus

AGID to search for antibodies to the gp 135 and p 30 common to the viruses of caprine arthritis encephalitis (CAEV) and maedi visna (MV). The antigen comprised the WLC 1 strain of MV virus cultivated in continuous line cells of lamb choroid plexus.

Encephalomyocarditis virus (EMCV)

MSN on serum inactivated at 56°C for 30 minutes, with initial 1:2 dilution, using 100 TCID₅₀ of a reference strain of EMCV (Pirbright) and, as a substrate, a continuous line of African green monkey kidney cells (Vero). The neutralising titre is expressed as the reciprocal of the highest dilution of serum capable of fully neutralising 100TCID₅₀ of virus.

Brucellosis S-phase

Rapid agglutination with Weybridge 99 antigen coloured with Bengal Red (RBT), slow serum agglutination (SSA) and complement fixation test (CFT) by micromethod with antigen B99. Minimum starting dilution of the serum, respectively: undiluted, 30 UIA, 20 CEE.

Leptospira interrogans

Microagglutination test (MA) in micromethod for the following eight sero groups: australis (serovar bratislavaj, icterohaemorrhagiae (icterohaemorrhagiae and copenhageni). canicola (canicola), pomona (pomona). hebdomadis (hardjo), sarassovi (hyos), grip-

potyphosa (grippotyphosa), sejroe (saxkoebing). Titres $\geq 1:100$ were considered.

Mycobacterium paratuberculosis

AGID on undiluted serum and the specific soluble extract utilised for antigen at 10 mg/ml concentration.

Chlamydia psittaci

CFT in micromethod on initially diluted serum 1:16 and using specific corpusculated antigen (Rickettsia Dept. WHO Centre of Bratislava, Slovakia). Titres $\geq 1:16$ were considered positive.

Coxiella burnetii

CFT in micromethod on initially diluted serum 1:8 and using specific corpusculated antigen (Rickettsia Dept. WHO Centre of Bratislava, Slovakia). Titres $\geq 1:8$ were considered positive.

Rickettsia mooseri

CFT in micromethod on initially diluted serum 1:8 and using specific soluble antigen

(Rickettsia Dept. WHO Centre of Bratislava, Slovakia). Titres $\geq 1:8$ were considered positive.

Rickettsia conori

CFT in micromethod on initially diluted serum 1:8 and using a soluble antigen of the Spotted Fever group (Rickettsia Dept. WHO Centre of Bratislava, Slovakia). Titres $\geq 1:8$ were considered positive.

Toxoplasma gondii

Direct agglutination using the Toxo-Screen DA (BIO Mérieux) starting from serum diluted 1:64. Titres $\geq 1:64$ were considered positive.

RESULTS AND DISCUSSION

Positive values are presented in Table 1. All animals were negative for brucellosis, FMD, PI3, BLV, caprine arthritis-encephalitis and visna -maedi, paratuberculosis, chlamydia, Q-fever and rickettsiosis. In 7 free-ranging chamois, the serum neutralisation test

Table 1 - Results of serum examination of captive and wild chamois expressed as serum positivity over tested.

PATHOGENIC AGENT	TESTS	NATURE POS./TESTED	CAPTIVITY POS./TESTED
BHV-1	MSN	7/27	0/35
Virus PI3	IHA	0/27	0/35
Pesti virus	ELISA	0/27	6/35
Virus aftoso OAC	ELISA	0/27	0/35
BLV	AGID	0/27	0/35
<i>Lentivirus oviscaprini</i>	AGID	0/27	0/35
EMCV	MSN	5/27	3/35
<i>Brucellae abortus</i>	RBPT	0/27	0/35
	SAL		
	CFT		
<i>Leptospira interrogans</i>	MAT	2/27	4/35
<i>Myc. paratuberculosis</i>	AGID	0/27	0/35
<i>Chlamydia psittaci</i>	CFT	0/27	0/35
<i>Coxiella burnetii</i>	CFT	0/27	0/35
<i>Rickettsia mooseri</i>	CFT	0/27	0/35
<i>Rickettsia conori</i>	CFT	0/27	0/35
<i>Toxoplasma gondii</i>	DA	2/27	2/35

for BHV-1 showed antibodies at minimum titre tested of 1:4. This low positiveness, could be due to receptivity for the chamois to this disease as a result of possible contact with cattle in winter time. But at present there are no references to what should be considered the positive level for this species, so further information are required.

Cattle pesti virus antibodies were encountered at low titre (1:4 and 1:8) in 6 sera of which 5 belonged to animals from Bisegna Wildlife Area in Abruzzo National Park; around this enclosure contacts with some grazing cattle and sheep were possible. The antigen used in this test do not discriminate between BVD and BD antibodies, so it was not possible to establish which pathogenic agents were responsible for the serum reaction.

Regarding EMCV, positivity which ranged from 1:8 to 1:128 was found in 5 wild and 3 captive chamois. In Italy this virus is widespread in wild (Amaddeo *et al.*, 1991; Cardeti *et al.*, 1992) and domestic animals (Gualandi *et al.*, 1989). The positive results in both captive and wild chamois, seem to suggest that the virus exists in the study area and that the chamois is receptive to this agent.

Data from leptospirosis tests revealed antibodies in 6 sera: 3 for the Australis serogroup (titre 1:100), 1 for icterohaemorrhagiae (titre 1:200) and 2 for both at 1:200 titre. These levels were not indicative of active infection, but showed the presence of icterohaemorrhagiae in the Park area and especially Australis sera groups. Australis group serotypes are spreading in the environment as shown by their isolation in different species such as cattle (Autorino *et al.*, 1990) and dogs (Autorino *et al.*, 1994). It could have a pathogenic role for the chamois too. Regarding the toxoplasma antibodies, 2 free-living animals (titre 1:64 and 1:4096) and 2 kept in captivity in two wildlife areas in Abruzzo National Park (titre 1:64 and 1:4096) were positive. This positivity and the maintenance of high antibody titre (\geq 1:4096) at a distance of a year and half in the same animal transferred from free life in

Val di Rose to a wildlife area, confirm the receptivity of chamois to this agent. Its pathogenic role in the species is unknown.

CONCLUSION

Evaluation of the results obtained from free-range animals living in the Val di Rose area in Abruzzo National Park and others kept in wildlife areas, allow us to affirm that present health status is satisfactory. The low titres of positivity observed, associated with the lack of symptomatology related to the different pathologies, exclude active infection and suggest that there is only contact with different pathogenic agents. For 18 specimens additional samples were drawn, but there was no new positivity, or any significant increase in the antibody titre in positive animals. The 33 subjects examined and used for introduction (11 from the wild and 22 from wildlife areas) were free from diseases mentioned under Veterinary Police Regulations. It is extremely important to continue the controls which have been made up to now to detect possible modifications of health conditions which may impair the preservation of Abruzzo chamois population.

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