Praca oryginalna *Original paper*

Case-control studies on the occurrence of the neurologic form of caprine arthritis-encephalitis in Poland

JAROSŁAW KABA, MARIUSZ NOWICKI, MAŁGORZATA SOBCZAK-FILIPIAK*, LUCJAN WITKOWSKI, DOROTA NOWICKA, MICHAŁ CZOPOWICZ, OLGA SZALUŚ-JORDANOW**, EMILIA BAGNICKA***

Division of Infectious Diseases and Epidemiology, Department of Large Animal Diseases with the Clinic,
*Division of Animal Pathomorphology, Department of Pathology and Veterinary Diagnostics,
**Division of Infectious Diseases, Department of Small Animal Diseases with the Clinic, Faculty of Veterinary Medicine,
Warsaw University of Life Sciences – SGGW, Nowoursynowska 159c, 02-776 Warsaw,

***Institute of Genetics and Animal Breeding, Polish Academy of Sciences,
Jastrzębiec, Postępu 1, 05-552 Wólka Kosowska

Kaba J., Nowicki M., Sobczak-Filipiak M., Witkowski L., Nowicka D., Czopowicz M., Szaluś-Jordanow O., Bagnicka E.

Case-control studies on the occurrence of the neurologic form of caprine arthritis-encephalitis in Poland

Summary

Caprine arthritis-encephalitis (CAE) is a chronic multisystemic disease widespread in the Polish goat population. It is caused by caprine arthritis-encephalitis virus (CAEV). The CAEV infection can lead to many clinical manifestations including neurological signs. The objective of the study was to estimate the prevalence of the neurologic form of CAE in Poland. Case-control studies were based on data collected during three disease surveys carried out in a population of goats covered by a milk recording program. The data were collected in 1996, 2002 and 2007 in 72, 57 and 39 herds, respectively. ELISA test was applied to classify herds as seropositive and seronegative. No statistically significant relationship between the occurrence of neurological signs in adult goats and the presence of seropositive goats in a herd could be shown. Odds ratios for exposure calculated using the results of the studies from 2002 and 2007 (OR_{exp} = 0.633 and 0.513, respectively) were statistically insignificant ($\alpha = 0.05$). Neurological signs were observed significantly more often ($\alpha = 0.01$) in adult goats than in kids. The study provided serological and histopathological diagnosis of a case of the neurologic form of CAE in an 11-week-old kid with a typical clinical presentation. It is the first instance that the condition has been diagnosed by laboratory methods in Poland. The prevalence of the neurologic form of CAE in Poland seems to be very low. It was estimated that it occurs in at most 0.65% of herds per year and it does not seem to be of high clinical importance in Poland.

Keywords: caprine arthritis-encephalitis, epidemiology, neurological signs, goats

Caprine arthritis-encephalitis (CAE) is a chronic disease which occurs mostly in goats, with sheep being sporadically affected. It is caused by the caprine arthritis-encephalitis virus (CAEV), belonging to the family *Retroviridae*, genus *Lentivirus*. The closely related lentivirus is responsible for the occurrence of maedi-visna disease (MV) in sheep. Since clinical presentation is very similar in both diseases and cross-infections between these two small ruminant species have been described, both viruses are referred to as small ruminant lentiviruses, SRLVs (7). SRLVs produce a lifelong infection which remains dormant for a long time and eventually leads to assorted clinical and pathological manifestations. Although an infected animal may remain asymptomatic for its whole lifetime, usually one of the following presentations develops: arthritis, interstitial pneumonia, *mastitis*, progressive weight loss or neuropathy. Neurological signs are observed quite rarely (12, 15). The neurologic form of CAE occurs mainly in 1-6-month-old kids but in rare cases also can be encountered in adult goats (8). It begins with ataxia of the hind limbs, then slowly over

several weeks turns into tetraparesis and ends with paralysis and recumbency. Affected goats usually remain bright and alert as well as continue to eat and drink until the last stage of the disease when bedsores and secondary infections develop, causing natural death or, more often, being a ground for euthanasia. Other neurological signs such as depression, blindness, head tilt, torticollis and circling may also be observed. CAE has been recognized in Poland for many years and many goat herds are affected (6). Moreover, clinical signs characteristic for the neurologic form of CAE have been described in one herd infected with CAEV (11). However the neurologic form of CAE has never been diagnosed by laboratory methods in Poland.

The objective of the study was to estimate the prevalence of the neurologic form of CAE in Poland.

Material and methods

Herds. The study involved the population of goats covered by a milk recording program in Poland. Data for epidemiological analysis were obtained from three consecutive disease surveys carried out in 1996, 2002 and 2007.

Diagnosis of CAE. Serological tests were applied to establish the status of each herd (seropositive or seronegative) in all three surveys; moreover, only females older than 12 months were examined. Animals for the study were selected in a simple random way so that at least one seropositive animal could be detected with a level of confidence 0.95 and pre-assumed prevalence 10% (16). Calculations were performed in Win Episcope 2.0 (Epidecon). Serological tests were performed with ELISA (Chekit CAEV/MVV, Bommeli AG, Switzerland) and a herd was classified as seropositive when at least one female appeared positive in ELISA. In that manner 71 seropositive and 97 seronegative herds were selected (tab. 1).

Questionnaires. During each survey data on the date when a herd was established as well as on the occurrence of neurological signs in kids (< 12-month-old) and adults (> 12-month-old) on the day of a visit and within 5 previous years were collected in the form of a standardized interview from an owner or a caretaker. Herd inspection was performed each time, with special attention paid to neurological signs and the body condition of goats.

Necropsy. A kid in which neurological signs were noticed during herd inspection was euthanized and underwent routine pathological, microscopic and microbiological examination. Specimens from organs with prominent lesions were chosen for further microscopic evaluation, and were fixed in 10% buffered formalin and embedded in paraffin, then stained with hematoxilin and eosin (HE). Moreover fragments of the nervous system were stained with Klűver-Barer's method to display myelin. Fragments of the brain, lungs, kidneys and liver were taken for microbiological examination. The material was cultured on agar enriched with 5% sheep blood (Biomed, Lublin) and plates were incubated at 37°C in aerobic and anaerobic conditions for 72 hours.

Epidemiological and statistical analysis. Prevalence of the neurologic form of CAE in seropositive herds was calculated as a proportion of herds in which such cases had been observed during a 15-year-long period to population size expressed as herd-year at risk (16). Prevalence of the neurologic form of CAE in kids and adult goats was compared with the use of a test (8). The null hypothesis on equality of prevalences was verified at the level of significance $\alpha = 0.01$. The influence of serological status on the occurrence of neurological signs in a herd was evaluated according to general principles for case-control studies by calculation of odds ratio for exposition (OR_{exp}) in each age group and statistical interpretation was performed basing on analysis of confidence intervals (CI) at a level of confidence 0.95 (16). Calculations were performed in Win Episcope 2.0 (Epidecon).

Results and discussion

Studied population. Data from 1996, 2002 and 2007 were collected in 72, 57 and 39 herds, respectively (tab. 1). There were 71 CAE-seropositive herds (tab. 1) with a history of 1 to 5 years which yielded the total population size of 307 herds per year at risk.

Occurrence of neurological signs in kids. The occurrence of neurological signs in kids was revealed only in 2002. Two such cases were reported in 2 seropositive herds (tab. 1) (herds A and B) in 307 herds per year at risk, which made it possible to estimate the prevalence of neurological signs in seropositive herds at 0.65%. Since no neurological signs were observed in the remainder of the compared groups statistical analysis of the influence of CAEV infection on the occurrence of neurological signs in kids was not possible (16).

In herd A located in central Poland, an eleven week-old kid of the Polish White Improved breed manifesting neurological signs was found. The kid was in good body condition and alert, it continued to eat and drink but was unable to walk. Lower motoneuron tetraparesis, much more severe in the hind limbs was

Tab. 1. Occurrence of neurological signs in goats according to their age

Year of the study	Herds			Number of herds in which in goats			
	serological status	number		< 12-month-old neurological signs		> 12-month-old neurological signs	
				were observed	were not observed	were observed	were not observed
1996	seropositive		17	0	17	14	3
	seronegative	55		0	55	52	3
2002	seropositive		33	2	31	20	13
	seronegative	24		0	24	17	7
2007	seropositive		21	0	21	12	9
	seronegative	18		0	18	13	5
Total		97	71	2	166	128	40

prominent. As far as the owners were aware, the onset of hind limb paraparesis had taken place several weeks before. The signs tended to increase gradually and forelimbs also became included. Such a clinical course is typical for the neurologic form of CAE (8, 10). No similar manifestation could be noticed in any other goat. Adults as well as the remaining kids behaved normally. A serological check-up of the kid revealed antibodies to SRLV. Since CAE spreads within a herd mainly vertically from does to their offspring (12), the mother of the kid was also serologically examined and the result was positive. The kid was euthanized and necropsied. Considerable congestion of meninges and the brain (fig. 1), especially in the region of mesencephalon, along with pulmonary congestion and edema were the only significant gross lesions observed. Histopathological examination revealed the proliferation and aggregation of astrocytes, degeneration of ganglionic cells, as well as cellular inflammatory infiltrations (composed primarily of lymphocytes) around blood vessels (fig. 2) were revealed. Typical microscopic lesions in CAE are focal and can be observed in mesencephalon and distal parts of the central nervous system. They result from demyelination (5) and perivascular aggregates of inflammatory cells (4, 13). While no foci of demyelination could be found in the kid's brain, perivascular cellular inflammatory infiltrations in fragments of the mesencephalon were prominent. Interstitial pneumonia is another lesion frequently encountered in the course of CAE (5). Microscopic evaluation of the lungs showed congestion and edema as well as focal aggregations of inflammatory cells (fig. 3) and small foci of emphysema in interstitial tissue. No bacterial growth was demonstrated.

Differential diagnosis of the neurologic form of CAE should include enzootic ataxia due to copper deficiency (swayback), vertebral body or spinal cord abscesses as well as injuries and congenital abnormalities of the spinal cord or vertebrae column (2, 8, 14). No gross lesions of vertebrae column were found in the necropsy. Data collected from the goat caretakers did not indicate copper deficiency. Characteristic neurological signs were observed in one kid exclusively and herd inspection excluded any neurological problems in other animals, both adults and kids. All animals were in good condition and constant access to mineral and vitamin supplements was ensured. Copper deficiency is usually endemic and occurs in a number of

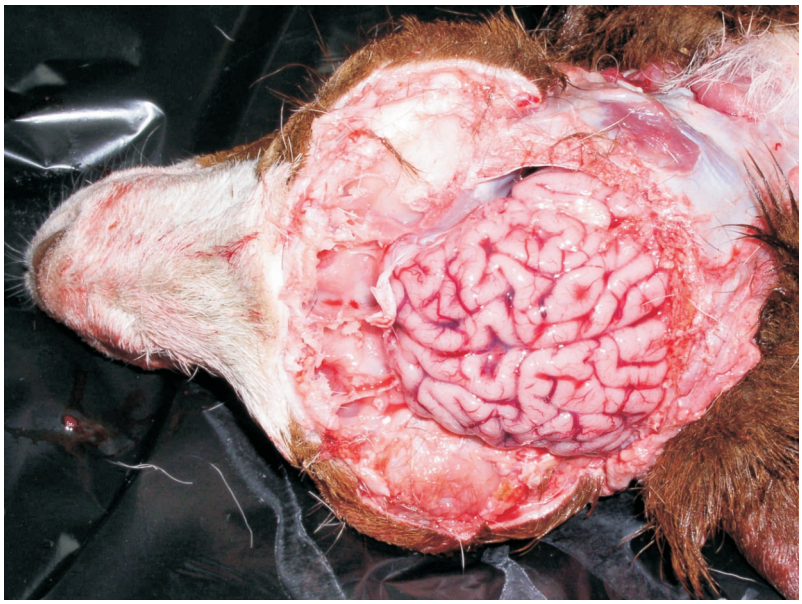

Fig. 1. Considerable congestion of the meninges and the brain

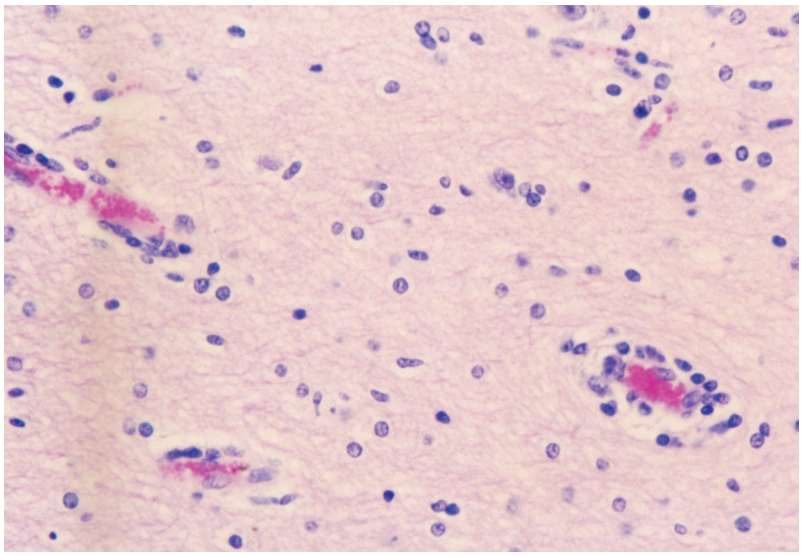

Fig. 2. Microscopic image of the brain: perivascular cellular inflammatory infiltrations (mostly lymphocytes) (HE staining, magnification 40×)

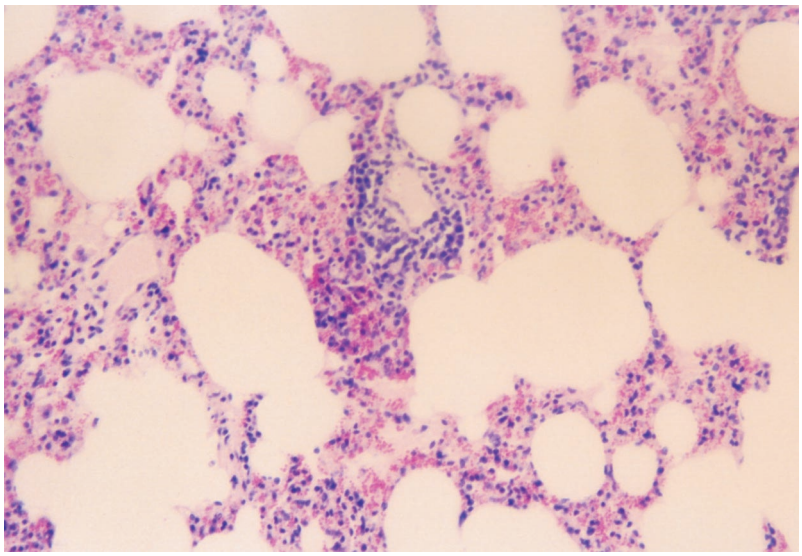

Fig. 3. Microscopic image of the lungs – interstitial pneumonia with lymphatic nodule formation (HE staining, magnification 20×)

animals at the same time (2, 3). The kid was born healthy, with no clinical signs typical for copper deficiency, such as diarrhea, anemia, muscle tremors or alterations in hair coat (1, 2). In the histopathological examination no typical lesions such as cerebellar atrophy or hypoplasia, liver fibrosis and necrosis, proliferation of bile ducts and presence of syderocytes were demonstrated (1-3). In conclusion the course of the disease, clinical manifestation as well as the results of serological tests and necropsy seem to confirm that it is a case of the neurologic form of CAE. This is the first disease case diagnosed by laboratory methods in Poland.

In herd B located in northeastern Poland no animal with neurological signs was found on the day of a visit. Nevertheless goat caretakers reported that such cases had occurred in a few goats during the previous two years. Paralyses of hind limbs and sometimes also forelimbs, torticollis and head tilt in kids as well as mastitis in females after first delivery had been observed many times in the past. Many adult goats manifested arthritis and emaciation, whereas poor body condition was prominent in kids on the day of inspection. Antibodies to CAEV were detected in the herd as well. Data collected during the visit confirmed earlier observations carried out on the same herd (11). All these allegations make it possible to presume that a neurologic form of CAE might have occurred in the herd. On the other hand it has to be stressed that the presumptive diagnosis was based only on retrospective data. As they are always less credible it cannot be ruled out that the poor body condition of both kids and adult goats as well as numerous cases of neurological signs might have been the manifestation of other diseases, for instance copper deficiency.

Occurrence of neurological signs in adult goats. Even though no neurological signs were noted in goats older than 12 months in any survey, interviews revealed that they had been frequently observed by goat caretakers in the past. They were reported in 128 herds (tab. 1) in 307 herds per year at risk, thus prevalence was 41.69%.

Statistical analysis of data collected in 1996 was not possible because of the too small size of groups (tab. 1) (16). Odds ratios for exposure calculated using results of the studies from 2002 and 2007 were statistically insignificant 0.633 (CI 0.206, 1.948) and 0.513 (CI 0.134, 1.970), respectively. Therefore no relationship between the presence of antibodies to CAEV in a herd and the occurrence of neurological signs could be demonstrated. There are many diseases in which neurological signs in adult goats may appear: listeriosis, enterotoxaemia and copper or B_1 vitamin deficiency belong to the most common diseases with a similar clinical manifestation (14) and could lead to the emergence of neurological signs in adult goats.

The prevalence of the neurologic form of CAE in seropositive herds. The prevalence of neurological signs in seropositive herds was 0.65% among kids and 41.69% among adult goats. These values were significantly different ($u_{emp} = 43.0$; $\alpha = 0.01$). No significant relationship between the occurrence of neurological signs and CAEV infection could be demonstrated in adult goats. In turn, among kids neurological signs were reported only from seropositive herds. The clinical manifestation observed in kids (progressive hind limb paresis) was characteristic for the neurologic form of CAE (8, 10). Serological and histopathological investigation of the kid from herd A seems to confirm the diagnosis of a neurologic form of CAE. In the case of herd B no laboratory tests could be performed to establish the definitive diagnosis but information collected from the interview with the owner and the serological examination of the herd are strongly suggestive of a neurologic form of CAE (11). Taking into account what was mentioned above the estimated prevalence of the neurologic form of CAE is 0.65%. It enables drawing a conclusion that the neurologic form of CAE seems to occur rarely and is clinically insignificant in Poland.

References

1. *Aupperle H., Schoon H. A., Frank A.*: Experimental copper deficiency, chromium deficiency and additional molybdenum supplementation in goats – pathological findings. Acta. Vet. Scand. 2001, 42, 311-321.
2. *Banton M. I., Lozano-Alarcon F., Nicholson S. S., Jowett P. L., Fletcher J., Olcott B. M.*: Enzootic ataxia in Louisiana goat kids. J. Vet. Diagn. Invest. 1990, 2, 70-73.
3. *Cordy D. R., Knight H. D.*: California goats with a disease resembling enzootic ataxia or swayback. Vet. Pathol. 1978, 15, 179-185.
4. *Gonzalez L., Gelabert J. L., Marco J. C., Saez de Okariz C.*: Caprine arthritis-encephalitis in the Basque country, Spain. Vet. Rec. 1987, 120, 102-109.
5. *Jubb K. V. F., Palmer N., Kennedy P. C.*: Pathology of Domestic Animals, Vol. 1, Academic Press Inc., San Diego 1991, p. 416.
6. *Kaba J., Ganter M., Bickhardt K., Prandota J.*: Prevalence of antibodies to caprine arthritis-encephalitis in breeding goats in Poland. Epidémiol. Santé Anim. 1997, 31-32, 11.
7. *Leroux C., Cruz J. C., Mornex J. F.*: SRLVs: a genetic continuum of lentiviral species in sheep and goats with cumulative evidence of cross species transmission. Curr. HIV Res. 2010, 8, 94-100.
8. *Norman S., Smith M. C.*: Caprine arthritis-encephalitis: review of the neurologic form in 30 cases. J. Am. Vet. Med. Assoc. 1983, 182, 1342-1345.
9. *Olech W., Wieczorek M.*: Zastosowanie metod statystyki w doświadczalnictwie zootechnicznym. Wydawnictwo SGGW, Warszawa 2002, pp. 152-153.
10. *Olsen J. C.*: EIAV, CAEV and other lentivirus vector systems. Somat. Cell. Mol. Genet. 2001, 26, 131-145.
11. *Platt-Samoraj A., Michalski M. M., Sobiech P., Mikulska-Skupień E.*: Przypadek zakażenia kóz wirusem zapalenia stawów i mózgu kóz w jednym ze stad w województwie warmińsko-mazurskim. (A case of caprine arthritis-encephalitis virus infection of goats in one of the herds in Warmia and Mazury voivodship). Medycyna Wet. 2003, 59, 802-804.
12. *Peterhans E., Greenland T., Badiola J., Harkiss G., Bertoni G., Amorena B., Eliaszewicz M., Juste R. A., Krassnig R., Lafont J. P., Lenihan P., Pétursson G., Pritchard G., Thorley J., Vitu C., Mornex J. F., Pépin M.*: Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Vet. Res. 2004, 35, 257-274.
13. *Sanna E., Sanna M. P., Vitali C. G., Renzoni G., Sanna L., Spano S., Rossi G., Leoni A.*: Proviral DNA in the brains of goats infected with caprine arthritis-encephalitis virus. J. Comp. Pathol. 1999, 121, 271-276.
14. *Smith M. C., Sherman D. M.*: Goat Medicine. Wiley-Blackwell, Ames 2009, 163-256.
15. *Straub O. C.*: Maedi-Visna virus infection in sheep. History and present knowledge. Comp. Immunol. Microbiol. Infect. Dis. 2004, 27, 1-5.
16. *Thrusfield M.*: Veterinary Epidemiology. Blackwell Science, Oxford 2007, 53-57, 228-288.

Corresponding author: Dr Jarosław Kaba, Nowoursynowska 159c, 02-776 Warsaw, Poland; e-mail: jaroslaw_kaba@sggw.pl