SHORT COMMUNICATION

Anaplasma phagocytophilum infection in thrombocytopenic dogs

Stefano De Arcangeli, Andrea Balboni, Federica Serafini, Mara Battilani and Francesco Dondi*

Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy.

Corresponding author at: Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Via Tolara di Sopra 50, 40064, Ozzano dell'Emilia (BO), Italy. Tel.: +39 051 2097317, Fax: +39 051 2097593, e-mail: f.dondi@unibo.it.

> Veterinaria Italiana 2018, **54** (1), 73-78. doi: 10.12834/VetIt.1070.5796.2 Accepted: 21.12.2016 | Available on line: 29.01.2018

Keywords

Anaplasmosis, Blood, Dog, Thrombocytopenia.

Summary

Anaplasma and *Ehrlichia* spp. are tick-transmitted bacteria of clinical relevance in European dogs. The diagnosis of infection is often difficult due to the wide spectrum of disease caused by them. During infection, reduction in platelet count is considered the most common haematological abnormality, frequently representing the sole alteration in asymptomatic dogs. In this study, the presence of bacteria belonging to the genera *Anaplasma* and *Ehrlichia* was investigated in Northern Italy in blood samples from 159 thrombocytopenic dogs using a polymerase chain reaction (PCR) assay amplifying a portion of the heat shock gene (*groEL*). Obtained amplicons were sequenced and analysed. Two dogs were positive for *A. phagocytophilum*, while *A. platys* and *E. canis* were not detected. None of the PCR-positive dogs were diagnosed at the time of hospital admission, even in the presence of clinical signs and clinicopathological abnormalities potentially related to *A. phagocytophilum* infection. Nucleotide sequence analysis showed that the 2 detected strains belonged to the cluster Europe 1 and were different from each other. This study confirms the presence of *A. phagocytophilum* infections in dogs of Northern Italy, causing clinical signs and laboratory abnormalities that could not be properly diagnosed and treated.

Infezione da Anaplasma phagocytophilum in cani trombocitopenici

Parole chiave

Anaplasmosi, Cane, Sangue, Trombocitopenia.

Riassunto

I batteri appartenenti ai generi *Anaplasma* ed *Ehrlichia* sono responsabili di infezioni nel cane trasmesse da zecche e di notevole rilevanza in Europa. L'infezione causa sintomatologia molto variabile con conseguenti difficoltà diagnostiche. La riduzione nel numero di piastrine circolanti è l'alterazione ematologica più comune e spesso rappresentata l'unico reperto riscontrabile nei cani sintomatici. In questo studio, la presenza di batteri appartenenti ai generi *Anaplasma* and *Ehrlichia* è stata indagata in Nord Italia nei campioni di sangue di 159 cani trombocitopenici utilizzando una metodica di PCR (reazione a catena della polimerasi) che amplifica un tratto del gene *heat shock (groEL)*. Gli ampliconi ottenuti sono stati sequenziati e analizzati. Due cani sono risultati positivi per *A. phagocytophilum*, mentre non sono state rilevate positività per *A. platys* ed *E. canis*. A nessuno dei cani risultati positivi in PCR era stata precedentemente diagnosticata l'infezione da *A. phagocytophilum*, pur presentando sintomi clinici o anomalie clinico-patologiche ad essa potenzialmente riferibili. L'analisi delle sequenze nucleotidiche dei 2 ceppi identificati ha permesso di correlarli al cluster Europe 1. Questo studio conferma che *A. phagocytophilum* è presente nei cani in Nord Italia ed è responsabile di forme cliniche non adeguatamente diagnosticate e curate.

Bacterial species belonging to the genera Anaplasma and Ehrlichia (family Anaplasmataceae) obligate are gram-negative, intracellular alphaproteobacteria that replicate in the cytoplasm of eukaryotic cells (Dumler et al. 2001). In particular, A. phagocytophilum, A. platys, and E. canis are tick-transmitted parasites of great importance to canine health in Europe. During infection, they form colonies called morulae in monocytes, granulocytes, and platelets (Allison and Little 2013). Dogs infected by these pathogens can develop a wide spectrum of disease, ranging from subclinical infection to potentially life-threatening illness characterized by fever, lethargy, anorexia and, in most severe cases, bleeding diathesis. Reduction in platelet count is considered the most common haematological abnormality during Anaplasma and Ehrlichia infection in dogs, frequently representing the sole alteration in asymptomatic cases (Little 2010, Allison and Little 2013). Nevertheless, data on the relevance of these infections as causes of thrombocytopenia are lacking.

The aim of the present study was to investigate the presence of bacteria belonging to the genera *Anaplasma* and *Ehrlichia* in blood samples from thrombocytopenic dogs.

The study was conducted in a veterinary teaching hospital, in Northern Italy, on pet dogs. All dogs referred to the veterinary hospital during a 19-month period (January 2013 - July 2014) which underwent ethylenediaminetetraacetic acid (EDTA)-blood sampling for complete blood count (CBC) (ADVIA 2120, Siemens Healthcare Diagnostics, Tarrytown NY, USA) and had thrombocytopenia, were included in the study. Thrombocytopenia was defined as a platelet count (PLT) of less than 160,000 platelets/ µL or less than 75,000 platelets/µL in subjects with evidence of platelet clumps on microscopic examination of a blood smear. Data related to signalment, clinical presentation, and laboratory findings (in particular the PLT values) of sampled dogs were recovered from medical records. Genomic DNA extraction from stored (-20 °C) EDTA-blood samples was carried out using the NucleoSpin Tissue Mini Kit (Macherey-Nagel, Düren, Germany). Diagnosis of infection was performed using a previously described polymerase chain reaction (PCR) assay (Barber et al. 2010). The 2 degenerate primers used in the present study, groEL-643s (5'-ACT GAT GGT ATG CAR TTT GAY CG-3') and groEL-1236as (5'-TCT TTR CGT TCY TTM ACY TCA ACT TC-3'), were able to detect DNA from all known Anaplasma spp. and *Ehrlichia* spp. by amplifying an approximately 600 bp fragment of the heat shock gene (groEL). The PCR assay was performed as previously described (Dondi et al. 2014). To confirm the reproducibility of the results, DNA extraction and PCR were repeated for the samples tested positive.

The obtained amplicons were purified and sequenced directly using both forward and reverse primers. The assembled nucleotide sequences were analysed using the BLAST web interface¹. Multiple alignments between obtained and reference sequences available from GenBank were generated using the ClustalW method implemented in BIOEDIT sequence alignment editor version 7.2.5. Phylogenetic relationships of obtained sequences with A. phagocytophilum reference sequences were evaluated using the maximum likelihood method implemented on MEGA version 6.0.6. Kimura 2-parameter nucleotide substitution model with gamma distribution was used. Bootstrap values were determined by 1000 replicates to assess the confidence level of each branch pattern and values > 50% were reported. The following reference A. phagocytophilum strains detected in several hosts from various parts of the world were obtained from GenBank and included in the molecular analysis: America lineage, accession numbers: AF172163; AY219849: AY848749; AY848750; AY848751; AY848752; DQ680012; Europe1 lineage, accession numbers: U96735; AY281849; AF033101; AF478563; AF482760; AF548386; AY529490; EU381150; EU982549; EU381151; EU381152; GQ452227; HM057224; KF778380; Europe2 lineage, accession AY220468; numbers: EU552912; EU552915; EU552919; EU552921; EU552923; Europe3 lineage, accession numbers: AF383227; AF478561; AY281818; EU552922.

Results were analysed using descriptive statistics. Data comparison among subgroups (breed, sex, age, and geographical origin) was performed by chi-square test; statistical significance was set at P < 0.05.

One hundred fifty-nine dogs were included in the study, and EDTA-blood samples were tested by PCR. DNA amplification products of the expected size were obtained from the blood samples of 2 dogs (862/2014 and 901/2014), producing an infection rate of 1.26%. Positive results were obtained by repeating the DNA extraction and PCR on blood samples of the 2 dogs. The assembled nucleotide sequences obtained for dogs 862/2014 and 901/2014 were 561 bp in length, and BLAST analysis allowed us to align them with the reference sequences of A. phagocytophilum (GenBank accession numbers: KT970678 and KT970679). Nucleotide alignment showed differences between the 2 detected strains (Annex 1, Supplementary Figure 1). The nucleotide sequences of 862/2014 and 901/2014 showed an identity of 99% between them and an identity of 99.5% with several reference strains detected in various hosts and countries (Dog/ IT/EU982549, Dog/SI/EU381150, Dog/SI/EU381151,

¹ http://blast.ncbi.nlm.nih.gov/Blast.cgi.



Figure 1. *Maximum likelihood tree based on the* gro*EL gene alignment.* Phylogenetic relationships were evaluated using the maximum likelihood method implemented on MEGA version 6.0.6. Kimura 2-parameter nucleotide substitution model with gamma distribution was used. Bootstrap values were determined by 1000 replicates to assess the confidence level of each branch pattern and values > 50% were reported. *A. phagocytophilum* reference strains were named with: host species, acronym of nation, and GenBank accession number. Highlighted in black: sequences generated in this study. In bold: reference strain 393 (KF778380) identified in a dog in the Veterinary Teaching Hospital of the University of Bologna, Italy, in 2012.

Goat/CH/GQ452227, Horse/CH/U96735, Horse/ DE/AF482760, Horse/SE/AY529490, Human/SI/ AF033101, Tick/DE/AY281849, Tick/SI/EU381152), including an *A. phagocytophilum* strain previously identified in a dog in the Veterinary Teaching Hospital of the University of Bologna, Italy (Dog/IT/ KF778380) (Dondi *et al.* 2014). The bacteria strains 862/2014 and 901/2014 showed 2 peculiar amino acid substitutions (data not shown). Phylogenetic analysis revealed 4 main clusters, supported by significant bootstrap values, consistent with the accepted nomenclature (Figure 1); 862/2014 and 901/2014 formed 2 independent monophyletic branches into cluster Europe 1. No significant difference in *A. phagocytophilum*-positive subjects between subgroups was detected.

In this study, the blood samples of 2 out of 159 thrombocytopenic dogs were PCR-positive for *A. phagocytophilum*. No dogs positive for *A. platys* or *E. canis* were detected. Dog 901/2014 showed no specific signs of *A. phagocytophilum* infection, with the exception of thrombocytopenia; whereas dog 862/2014 had clinical signs and clinicopathological abnormalities potentially related to A. phagocytophilum infection, such as anaemia, increased hepatic enzymes, hypoalbuminemia, thrombocytopenia, positive Coombs test, and abortion. Nevertheless, anaplasmosis had not been suspected at the time of hospitalization in either dog, and neither had been treated with specific therapy. Data on the prevalence of Anaplasma and Ehrlichia infections in Northern Italy are lacking. Moreover, comparison between the data obtained in this study and those described in previous epidemiological investigations is difficult due to the different inclusion criteria and diagnostic tools used. Nevertheless, the identification of 2 dogs infected by A. phagocytophilum and the absence of dogs infected by E. canis is in contrast with other epidemiological studies conducted in Italy, which have detected a high prevalence of E. canis and no cases of A. phagocytophilum infection (Solano-Gallego et al. 2006, Trotta et al. 2009). However, a case of A. phagocytophilum infection in a dog had already been reported in the province of Bologna (Dondi et al. 2014). Contrarily, the absence of A. platys in blood samples from dogs is in agreement with a previous molecular survey performed in the same geographic area (Trotta *et al.* 2009), suggesting a low circulation of this bacterium in Northern Italy. The discrepancies between *A. phagocytophilum* and *E. canis* infection rates evidenced in this study and those of other studies may be due to the choice of thrombocytopenia as the only clinicopathological finding used as inclusion criteria in the present study. In this way, a greater number of dogs infected by *A. phagocytophilum* may have been selected because this bacterium is often responsible for asymptomatic infections in dogs that show only thrombocytopenia (Egenvall *et al.* 2000, Little 2010).

In conclusion, this study allows us to confirm the presence of *A. phagocytophilum* infections in dogs of Northern Italy, causing clinical signs and laboratory abnormalities that could not be properly diagnosed and treated. Furthermore, even if the number of infected dogs on the total number of thrombocytopenic dogs analysed is relatively low, our study confirm that *A. phagocytophilum* should be considered as a potential cause of thrombocytopenia in dogs.

References

- Allison R.W. & Little S.E. 2013. Diagnosis of rickettsial diseases in dogs and cats. Vet Clin Pathol, 42, 127-144.
- Barber R.M., Li Q., Diniz P.P., Porter B.F., Breitschwerdt E.B., Claiborne M.K., Birkenheuer A.J., Levine J.M., Levine G.J., Chandler K., Kenny P., Nghiem P., Wei S., Greene C.E., Kent M., Platt S.R., Greer K. & Schatzberg S.J. 2010. Evaluation of brain tissue or cerebrospinal fluid with broadly reactive polymerase chain reaction for *Ehrlichia, Anaplasma*, spotted fever group *Rickettsia*, *Bartonella*, and *Borrelia* species in canine neurological diseases (109 cases). J Vet Intern Med, **24**, 372-378.
- Dondi F., Russo S., Agnoli C., Mengoli N., Balboni A., Alberti A. & Battilani M. 2014. Clinicopathological and molecular findings in a case of canine *Anaplasma phagocytophilum* infection in Northern Italy. *Scientific World Journal*, 810587.
- Dumler J.S., Barbet A.F., Bekker C.P., Dasch G.A., Palmer G.H., Ray S.C., Rikihisa Y. & Rurangirwa F.R. 2001. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*,

Cowdria with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol*, **51**(Pt 6), 2145-2165.

- Egenvall A., Lilliehöök I., Bjöersdorff A., Engvall E.O., Karlstam E., Artursson K., Heldtander M. & Gunnarsson A. 2000. Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs. *Vet Rec*, **146**, 186-190.
- Little S.E. 2010. Ehrlichiosis and anaplasmosis in dogs and cats. *Vet Clin North Am Small Anim Pract*, **40**, 1121-1140.
- Solano-Gallego L., Trotta M., Razia L., Furlanello T. & Caldin M. 2006. Molecular survey of *Ehrlichia canis* and *Anaplasma phagocytophilum* from blood of dogs in Italy. *Ann N Y Acad Sci*, **1078**, 515-518.
- Trotta M., Fogliazza A., Furlanello T. & Solano-Gallego L. 2009. A molecular and serological study of exposure to tick-borne pathogens in sick dogs from Italy. *Clin Microbiol Infect*, **15**(Suppl 2), 62-63.

Annex 1

Publisher: Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' | **Journal:** Veterinaria Italiana | **Article Type:** Reasearch Article | **Volume:** XX; **Issue:** X; **Year:** 2018; **doi:** 10.12834/Vetlt.1070.5796.2

Supplementary Figure 1. *Nucleotide sequence alignment of partial* gro*EL gene between obtained and reference sequences.* Multiple alignments between obtained and reference sequences available from GenBank were generated using the ClustalW method implemented in BIOEDIT sequence alignment editor version 7.2.5. Alignment was generated with sequences of 437 bp in length, because not all the reference sequences covered the 561 bp of the two sequences obtained in this study. The ruler at the top shows the nucleotide positions. Dots represent nucleotide positions identical to the upper reference sequence. —cont'd



Veterinaria Italiana 2018, 54 (1), 73-78. doi: 10.12834/Vetlt.1070.5796.2

Annex 1

Publisher: Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' | **Journal:** Veterinaria Italiana | **Article Type:** Reasearch Article | **Volume:** XX; **Issue:** X; **Year:** 2018; **doi:** 10.12834/Vetlt.1070.5796.2

Supplementary Figure 1. *Nucleotide sequence alignment of partial* gro*EL gene between obtained and reference sequences.* Multiple alignments between obtained and reference sequences available from GenBank were generated using the ClustalW method implemented in BIOEDIT sequence alignment editor version 7.2.5. Alignment was generated with sequences of 437 bp in length, because not all the reference sequences covered the 561 bp of the two sequences obtained in this study. The ruler at the top shows the nucleotide positions. Dots represent nucleotide positions identical to the upper reference sequence. —cont'd

	280 290 300 310 320 330 340 350 360
Horse/SE/ay529490	GOTACTE AS A SCHOTZ ACCE TO SACE A SCHOLD AT THE AT ACCESS TO THE AT ACCESS TO THE A BASE AT ACCESS TO THE A
Horse/CH/U96735	360
Horse/DE/AF482760	360
Tick/DE/AY281849	360
Horse/SardiniaTT/12848749	
Dog/SardiniaTT/AV848751	6 b T 360
Dog/SardiniaTT/AV848750	
Dog/SardiniaTT/AV848752	
Dog/TT/EU982549	360
Tick/ST/EU381152	360
Dog/ST/FU381150	360
Dog/SI/E0301151	360
Human/ST/AF033101	360
Red deer/ST/3F478563	360
Sheep /NO /12548386	C 260
Human /IISE /EF172163	6 b T 360
Dog/USB/BY219849	
Tick /TT /FU552912	c T 360
Tick/IT/E0552919	360
Goat /CH /GO452227	260
Cat /USE /D0680012	6 b T 360
Red deer/ES/HM057224	360
Dog/17/KF778380	360
Tick/ITT/EU552922	360
Tick/DF/20281818	360
Ros deer/DE/3E383227	
Roedeer/ST/3F478561	T 360
mick/ITT/EU552921	360
Roedeer/CZ/3V220468	e 360
mick/17/20552915	a
Tick/IT/E0552923	a 260
862/2014 87970678	e 360
901/2014 10970679	T 260
Horse/SE/AY529490 Horse/CH/U96735	антеллятеллястся атволлантеткоттелентиче сало страдолае страдолае страдолае и 437 437
Namaa (DD (30480760	
HOISE/DE/AE402/00	
Tick/DE/AY281849	
HOISE/DE/AF482/80 Tick/DE/AY281849 Horse/SardiniaIT/AY848749	437
Tick/DE/AY281849 Horse/SardiniaIT/AY848749 Dog/SardiniaIT/AY848751	
Tick/DE/AY281849 Horse/SardiniaIT/AY848749 Dog/SardiniaIT/AY848751 Dog/SardiniaIT/AY848750	437 437
Tick/DE/AY281849 Horse/SardiniaIT/AY848749 Dog/SardiniaIT/AY848751 Dog/SardiniaIT/AY848750 Dog/SardiniaIT/AY848752	
Norse/JB/AF62/160 Tick/DE/AY281849 Horse/SardiniaIT/AY848749 Dog/SardiniaIT/AY848750 Dog/SardiniaIT/AY848752 Dog/IT/EU982549	
NGIBY/D/AF42100 Tick/DE/AY281849 Horse/sardiniaTT/AY848759 Dog/sardiniaTT/AY848750 Dog/sardiniaTT/AY848750 Dog/TT/EU982549 Tick/ST/EU381152	437 437
NoIse/DE/Are2.00 Tic//DE/Ar281849 Horse/SardiniaIT/AY848750 Dog/SardiniaIT/AY848750 Dog/SardiniaIT/AY848752 Dog/SardiniaIT/AY848752 Dog/SI/EU381152 Dog/SI/EU381150	
Noise/Us/Attaciev Tick/DS/Ay281849 Horse/SardiniaT/Ay848751 Dog/SardiniaT/Ay848750 Dog/SardiniaT/Ay848752 Dog/Tf/EV9262549 Tick/SJ/EU381152 Dog/SI/EU381150	437 437
NOIse/06/A442.80 Noise/SardiniaTT/AY848751 Dog/sardiniaTT/AY848751 Dog/sardiniaTT/AY848752 Dog/srdiniaTT/AY848752 Dog/sr/EU381152 Dog/sr/EU381151 Human/SI/AF033101 Human/SI/AF033101	
NOIse/DB/AF42180 NOIse/DB/AF42180 Noise/SardiniaIT/X048750 Dog/SardiniaIT/X048750 Dog/SardiniaIT/X048752 Dog/SardiniaIT/X048752 Dog/SI/KU038152 Dog/SI/KU038150 Dog/SI/KU038151 Numan/SI/KU038153	437 437
Mole/DD/A42180 Mole/SardiniaT/XH48743 Dog/sardiniaT/XH48730 Dog/sardiniaT/XH48750 Dog/sardiniaT/XH48750 Dog/srdiniaT/XH48750 Dog/sr/RU38152 Dog/sr/RU38152 Dog/sr/RU38151 Numan/Sr/RF033101 Red_der/sr/R2633 Shep/No/R263436	
NO18/02/02/04/442180 NO18/02/04/442180 Norse/SardiniaT7/X048750 Dog/sardiniaT7/X048750 Dog/sardiniaT7/X048750 Dog/sardiniaT7/X048750 Dog/s12/EU982549 Tick/s1/EU981150 Dog/s12/EU981150 Dog/s12/EU981150 Numan/S1/X0730101 Red_dee/s12/X047853 Sheep/N0/XF481868 Buman/U52/AF121263	
NOIse/DD/AP42180 NOIse/DD/AP42180 Noise/SardiniaTT/AP48735 Dog/SardiniaTT/AP48755 Dog/SardiniaTT/AP48750 Dog/SardiniaTT/AP48750 Dog/SardiniaTT/AP48750 Dog/ST/EU38152 Dog/ST/EU38151 Numan/ST/AP03101 Sheey/NO/AP548386 Numan/US3/AP172163 Dog/US3/AP172163	437 437 437 .
NO154/06/A442180 NO154/06/A442180 Norse/SardiniaT7/A7848739 Dog/SardiniaT7/A7848750 Dog/SardiniaT7/A7848750 Dog/SardiniaT7/A7848750 Dog/S1/EU982152 Dog/S1/EU983150 Dog/S1/EU983150 Dog/S1/EU983150 Numan/S1/A784386 Numan/S1/A477553 Sheep/No/AF43836 Numan/S1/A4772163 Dog/US/A78172163 Dog/US/A78172163	437 437
NOISE/DD/AP42180 NOISE/DD/AP42180 Noise/Sacdiniatr/AP48743 Dog/Sacdiniatr/AP48756 Dog/Sacdiniatr/AP48756 Dog/Sacdiniatr/AP48756 Dog/Sacdiniatr/AP48756 Dog/Sz/EU38152 Dog/Sz/EU38155 Dog/Sz/EU38155 Sheep/NO/AP548386 Numan/USA/AP472643 Sheep/NO/AP548386 Numan/USA/AP212849 Tack/TZ/EU552312	437 437
NOIS#/DE/AF42180 NOIS#/DE/AF42180 Norse/SardiniaTF/XP48175 Dog/SardiniaTF/XP48175 Dog/SardiniaTF/XP48175 Dog/SardiniaTF/XP48175 Dog/S1/EU38152 Dog/S1/EU38152 Dog/S1/EU38152 Dog/S1/EU38152 Numan/S1/AF48356 Numan/S1/AF48386 Numan/S1/AF48386 Numan/S1/AF48386 Tick/TJ/EU552312 Tick/TJ/EU552312 Tick/TJ/EU552312	437 437
NOISE/DD/AP42180 NOISE/DD/AP42180 Noise/Sacdiniatr/AP48743 Dog/Sacdiniatr/AP48750 Dog/Sacdiniatr/AP48750 Dog/Sacdiniatr/AP48750 Dog/Sacdiniatr/AP48750 Dog/Sz/EU38152 Dog/Sz/EU38151 Numan/Sz/AP73543 Sheep/NO/AF548386 Numan/Sz/AP73543 Sheep/NO/AF548386 Numan/Sz/AP721843 Dog/Sz/EU3845 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JT/FEU552912 Tick/JSZ	437 437
NO154706742180 NO154706742180 Norse/SardiniaT774788750 Dog/SardiniaT774788750 Dog/SardiniaT774788750 Dog/SardiniaT774788750 Dog/SardiniaT774788750 Dog/SardiniaS1152 Dog/S1728038152 Dog/S1728038153 Numan/S1747933101 Red_deer/S17487853 Sheey/No/A7843886 Numan/VS3/AF172183 Dog/VS3/AF172183 Dog/VS3/AF172183 Tick/T7780552912 Tick/T7780552912 Cat/US3/D0680012 Red_deerZS7/M857224	437 437
NOISE/DD/AP42180 NOISE/DD/AP42180 NOISE/SacdiniatX/XV48743 Dog/SacdiniatX/XV48750 Dog/SacdiniatX/XV48750 Dog/SacdiniatX/XV48750 Dog/Siz/EU38152 Dog/Siz/EU38152 Dog/Siz/EU38153 Shep/NO/XF548386 Numan/USA/XV21849 Dog/Siz/EU38153 Shep/NO/XF548386 Numan/USA/XV21849 Tick/T/T/EU52312 Tick/T/T/EU52313 Cat/USA/D682227 Cat/USA/D6852227 Cat/USA/D6857224 Dog/TZ/AF73630	437 437 437 .
NOIse/DD/Ar42:80 Noise/SardiniaT7/XP48759 Dog/SardiniaT7/XP48759 Dog/SardiniaT7/XP48750 Dog/SardiniaT7/XP488750 Dog/SardiniaT7/XP488750 Dog/SardiniaT7/XP488750 Dog/SardiniaT7/XP488750 Dog/Sard/N03151 Red_deer/SarJAP478563 Shep/No/XP47858386 Human/SI/AP478563 Shep/No/XP4784386 Human/SI/XP4784386 Human/SI/XP4784386 Car/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386 Dog/US/XP4784386	437 437
NOT#YDD/AP42180 NOT#YDD/AP42180 NOT#YJZACINIAIT/XV48473 Dog/saciniaitT/XV484730 Dog/saciniaitT/XV484730 Dog/saciniaTY/XV48730 Dog/sr/U098152 Dog/sr/U098152 Dog/sr/U098153 Dog/sr/U098153 Sheep.NO/AF548386 Numan/U5A/AP472543 Sheep.NO/AF548386 Tick/T/T/E052312 Tick/Tr/E052312 Cat/USA/D652271 Cat/USA/D652271 Cat/USA/D652271 Cat/USA/D652271 Cat/USA/D652271 Cat/USA/D652271 Cat/USA/D652271	437
NOISE/DE/AR42180 NOISE/DE/AR42180 Noise/SardiniaTT/XV84873 Dog/SardiniaTT/XV84873 Dog/SardiniaTT/XV848750 Dog/SardiniaTT/XV848750 Dog/SardiniaTT/XV848750 Dog/SardiniaTT/XV848750 Dog/SardiniaTT/XV848750 Dog/SardiniaS151 Numan/SJ/RF03101 Red_dear/SJ/RF03503 Red_dear/SJ/RF03503 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552312 Tick/TT/RU552322 Tick/DE/AS218188 Red_dear/SS/RHS57224 Dog/Tr/RU552322 Tick/DE/AS21818	437 437
NOISE/DE/AP42180 NOISE/DE/AP42180 Norse/SardiniaT7/XP48175 Dog/SardiniaT7/XP48175 Dog/SardiniaT7/XP48175 Dog/SardiniaT7/XP48175 Dog/SardiniaT7/XP48175 Dog/S1/RU38152 Dog/S1/RU38152 Dog/S1/RU38152 Dog/S1/RU38152 Numan/S1/AP47853 Sheey/NO/AF483856 Numan/S1/AP47853 Sheey/NO/AF483856 Numan/S1/AP47853 Tick/T7/RU552912 Tick/T7/RU552912 Tick/T7/RU552923 Tick/T7/RU55292 Tick/T7/RU55292	437
NOISE/DD/AP42180 NOISE/DD/AP42180 Noise/SardiniaTT/XV48473 Dog/sardiniaTT/XV48473 Dog/sardiniaTT/XV484730 Dog/sardiniaTT/XV484730 Dog/sardiniaTT/XV484730 Dog/sr/EU38152 Dog/sr/EU38152 Dog/sr/EU38153 Numan/Sr/AP03103 Shep/NO/AP548386 Numan/USA/AF172163 Dog/USA/XT/8755391 Tick/TT/EU552912 Tick/TT/EU552912 Tick/TT/EU552912 Tick/TT/EU52218489 Tick/TT/EU5218489 Tick/TT/EU5218489 Tick/TT/EU5218489 Tick/TT/EU5218489 Tick/TT/EU5218489 Tick/TT/EU5218489 Tick/TT/EU52218 Dog/Tr/KF18300 Tick/CA/X4748561 Tick/TT/EU52222 Tick/TT/EU52222 Tick/TT/EU522221 Tick/TT/EU52221 Dog/Tr/KF18300 Tick/TT/F47478561 Tick/TT/F47478561 Tick/TT/F47478561 Tick/TT/F6752821	437
NO154/DE/AT42180 NO154/DE/AT42180 Norse/SardiniaT7/XF48750 Dog/SardiniaT7/XF48750 Dog/SardiniaT7/XF48750 Dog/SardiniaT7/XF48750 Dog/SardiniaT7/XF48750 Dog/SardiniaT7/XF48750 Dog/SardiniaT51 Numan/S1/AF033101 Red_deer/S1/AF033101 Red_deer/S1/AF03501 Tick/T7/R0552912 Tick/T7/R0552912 Tick/T7/R0552912 Tick/T7/R0552921 Tick/T7/R0552921 Tick/T7/R0552921 Tick/T7/R0552921 Red_deer/S1/AF035201 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF03521 Red_deer/S1/AF035251	437
NOISE/DD/AP42180 NOISE/DD/AP42180 Noise/SardiniaTT/XV48473 Dog/sardiniaTT/XV484730 Dog/sardiniaTT/XV484730 Dog/sardiniaTT/XV484730 Dog/sardiniaTT/XV484730 Dog/siz/RU33152 Dog/siz/RU33151 Numan/Si/RV33101 Red_der/fi/RV71543 Shep/NO/RF48386 Numan/USJ/RV71543 Shep/NO/RF48386 Numan/USJ/RV71543 Shep/NO/RF48386 Numan/USJ/RV71543 Shep/NO/RF48386 Tick/T/TR055315 Goat/CH/Qd52227 Goat/VSJ/R085312 Tick/T/TR055323 Tick/ZT/RU553232 Tick/ZT/RU553231 Red_der/fS/RH857224 Dog/siz/RV785321 Tick/ZT/RU553232 Tick/ZT/RU553232 Tick/ZT/RU553231 Red_der/DS/RH857224 Dog/Siz/RV7853231 Red_der/DS/RV853232 Tick/ZT/RU553232 Tick/ZT/RU553231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Reder/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7853231 Red/Siz/RV7855333 Red/Siz/RV	437
NOISA/DA/A42180 NOISA/DA/A42180 Norsa/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT7/XV848730 Dog/SardiniaT51 Numan/SI/A47383180 Human/SI/A47383180 Human/SI/A473853 Shep/No/A7843886 Human/SI/A4738532 Tick/T7/B0552313 Red_dex/ES/HM857224 Dog/TS/XV218483 Tick/T7/B0552323 Tick/T7/B0552323 Tick/T7/B0552323 Tick/T7/B0552323 Reodex/CS/A4788527 Reodex/CJ/X220468 Tick/T7/B0552315	437
Noise/DB/A42:80 Noise/Sardiniatr/AV848743 Dog/sardiniatr/AV848743 Dog/sardiniatr/AV848750 Dog/sardiniatr/AV848750 Dog/sardiniatr/AV848750 Dog/sardiniatr/AV848750 Dog/sr/zU03151 Dog/sr/zU03151 Numar/sr/A47854386 Numar/USA/A478543 Shep/no/AF548386 Numar/USA/A471543 Dog/USA/A7219449 Tick/T/FU052912 Tick/T/FU052912 Tick/T/FU052921 Tick/T/FU052921 Tick/DF/R8308 Tick/DF/R8308 Tick/DF/R8308 Needes/Tic/A783188 Red des/DF/AF3830 Tick/DF/R8308 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921 Tick/T/FU0552921	437