

Supplementary data

Meningoencephalitis with malacia caused by *Sarcocystis calchasi* in a rock pigeon in Japan

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Supplementary Table 1. Antibody used in immunohistochemistry

Antigen	Host species	Clonality	Clone name	Dilution	Antigen retrieval	Manufacture
Glial fibrillary acidic protein (GFAP)	Mouse	monoclonal	GA5	1:800	Microwaving ^a	Merck KGaA (Darmstadt, DE)
Cluster of differentiation 3 (CD3)	Rabbit	polyclonal	n.a.	1:100	Microwaving ^a	Abcam (Cambridge, UK)
Paired Box 5 (PAX5)	Mouse	monoclonal	24/PAX5	1:100	Autoclaving ^b	BD Biosciences (Franklin Lakes, USA)
Ionized calcium-binding adapter molecule 1 (Iba1)	Rabbit	polyclonal	n.a.	1:300	Autoclaving ^b	Wako (Osaka, JP)

Abbreviation: n.a, not applicable.

a: Microwaved at 90°C for 10 min in Dako target retrieval solution (pH 9.0).

b: Autoclaved at 121°C for 10 min in 10 mM citrate buffer (pH 6.0).

Supplementary Table 2. PCR primers for PCR

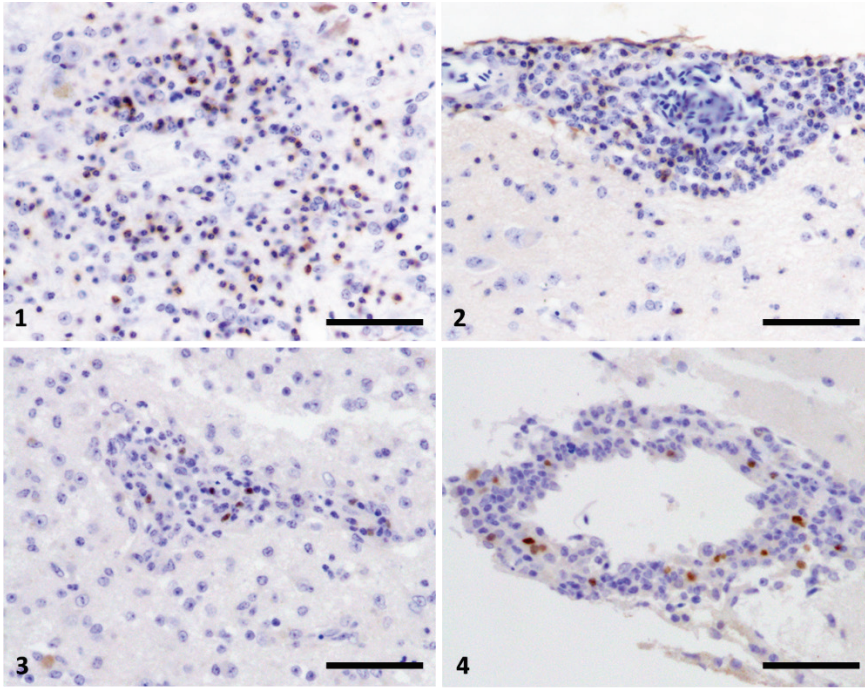
ID	Direction	Sequence (5'-3')	Amplicon size (bp)	Target site	References
<i>SAD2</i>	Forward	GGAAGCGATTGGAACC	350	28S rRNA	18
<i>SAD2</i>	Reverse	CCTTGGTCCGTGTTTCA		28S rRNA	18
<i>Sca1</i>	Forward	CTCCTTGCTCGAGAATGAACATGAG	276–300	ITS-1	11
<i>Sca2</i>	Reverse	GATCATCTTTTCGACGACAATATCG	474-498	ITS-1	11
<i>SNCa3</i>	Reverse	TCCAGAGAAGATCCCCTGGCTAC	389-498	ITS-1	11

Supplementary Table 3. Selected reports on *Sarcocystis* spp.-infected neuronal diseases in pigeons.

Species	Intermediates host and countries	Signs	Lesions	Cites	IHC	Reference No.
<i>Sarcocystis</i> spp.	Forty seven racing pigeons, Berlin, Germany	Depression, mild diarrhea, torticollis, opisthotonus, paralysis, and trembling	Granulomatous and necrotizing encephalitis (cuffing, gliosis) and malacia (brain stem and cerebellum)	Cerebrum, brain stem, and cerebellum	none	7
<i>Sarcocystis calchasi</i>	Five domestic pigeons*	Neurological disease	Multifocal lymphohistiocytic and necrotizing encephalitis (cuffing, gliosis, mononuclear cell infiltration)	Brain (including cerebrum)	CD3, PAX5, Iba-1, MHC II	12
<i>Sarcocystis calchasi</i>	One rock pigeon, Akihabara, Tokyo, Japan	Emaciation, diarrhea, torticollis, and ataxia	Multifocal inflammation (infiltration of mononuclear cells, cuffing, and meningitis)	Cerebrum and brain stem	CD3, PAX5, Iba-1	15
<i>Sarcocystis calchasi</i>	One White-winged Doves (<i>Zenaida asiatica</i>) and two Eurasian Collared Doves (<i>Streptopelia decaocto</i>), East-central Texas, USA	Neurologic signs	Severe, multifocal histiocytic and lymphoplasmacytic meningoencephalitis (gliosis, cuffing, meningitis, and plasmacyte/mott cell)	Cerebrum and brain stem	none	3
<i>Sarcocystis falcatula</i>	One free-ranging great horned owl (<i>Bubo virginianus</i>), Minnesota, USA	Unable to fly and severe head tilt to the left	Granulomatous meningoencephalitis (fibrinoid vascular necrosis, activated astrocyte) and malacia	Cerebrum, brain stem, and cerebellum	none	16
<i>Sarcocystis falcatula</i>	Three bald eagles (<i>Haliaeetus leucocephalus</i>) and one golden eagle (<i>Aquila chrysaetos</i>), Virginia, Indiana, and Minnesota, USA	Tremor, hock sitting, ventroflexion of the neck, weakness, and lethargy	Spongiform and multifocal glial nodules, gliosis, cuffing, and meningitis	Cerebrum and brain stem	none	17
<i>Sarcocystis halioti</i>	One Owl (<i>Athene noctua</i>), Michendorf, Gemany	Found dead	Granulomatous encephalitis	Cerebrum	none	6

* Experimental infection study: inoculated with 10^2 to 10^4 sporocysts, shed by an experimentally infected Northern goshawk (*A. g. gentilis*).

Abbreviations: Iba1, ionized calcium-binding adapter molecule 1; IHC, immunohistochemistry; MHC-II, major histocompatibility complex II; PAX5, paired box 5.



Supplementary Fig. 1. Infiltration of T lymphocytes into granulomatous lesions in the right cerebral hemisphere. CD3-positive T lymphocytes were observed in granulomatous lesions.

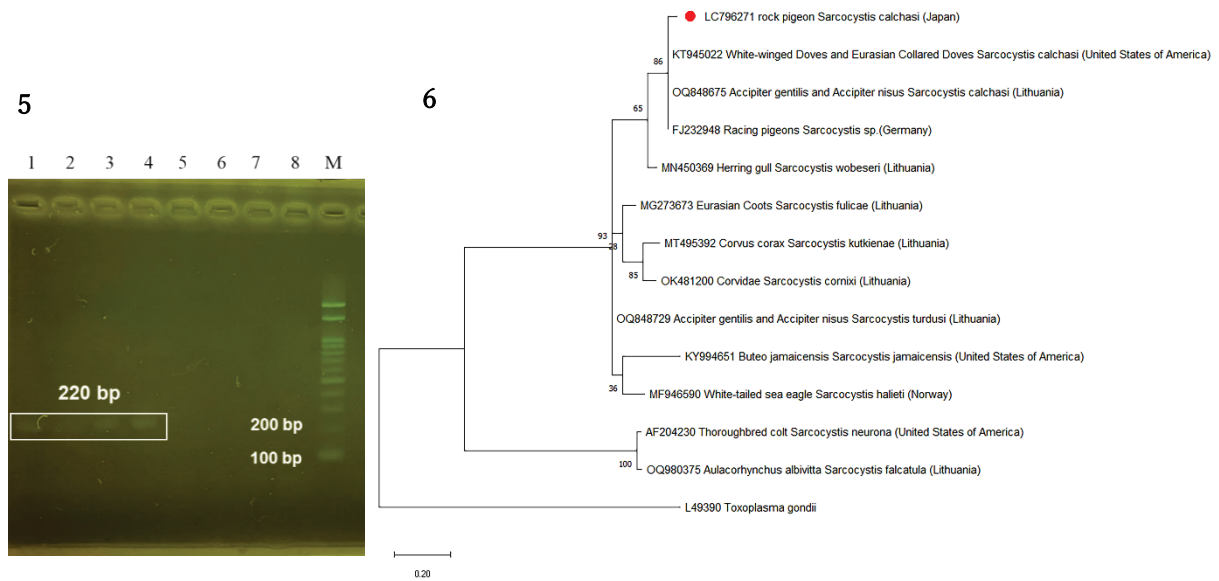
Immunohistochemistry for CD3, bar = 50 μ m.

Supplementary Fig. 2. Infiltration of T lymphocytes in the right cerebral hemisphere in patients with meningitis CD3-positive T lymphocytes were observed in subarachnoid spaces infiltrated by mononuclear cells. Immunohistochemistry for CD3, bar = 50 μ m.

Supplementary Fig. 3. Infiltration of B lymphocytes into granulomatous lesions in the right cerebral hemisphere. PAX5-positive B lymphocytes were scattered throughout the granulomatous lesions.

Immunohistochemistry for PAX5, bar = 50 μ m.

Supplementary Fig. 4. Infiltration of B lymphocytes in meningitis in the right cerebral hemisphere. PAX5-positive B lymphocytes were scattered in the subarachnoid spaces infiltrated by mononuclear cells. A leptomeningeal vessel was located in the subarachnoid space. Immunohistochemistry for PAX5, bar = 50 μ m.



Supplementary Fig. 5. Agarose gel electrophoresis (2%) showed the expected size (220 bp) of the PCR products from DNA using the primer pair Scal1/Scal2/SNCa3 for ITS-1. Lane M, DNA ladder; Lane 1, heart; Lane 2, medulla oblongata; Lane 3, midbrain; Lane 4, cerebrum; Lane 5, kidney; Lane 6, lungs; Lane 7, liver; Lane 8, spleen. PCR, polymerase chain reaction; DNA, deoxyribonucleic acid. Supplementary Fig. 6. Phylogenetic analysis of ITS-1 was performed using the maximum likelihood method with 1,000 bootstrap replications. Values of < 50% are not shown. The nucleotide sequences of the 28S rRNA detected in this study were contained within *Sarcocystis calchasi* and formed a separate branch within a group encompassing *Sarcocystis spp.* obtained from birds as intermediate or definitive hosts. *Toxoplasma gondii* was included in the control group. Bootstrap values are shown in the interior branch nodes, and scale bars indicate the number of substitutions per site. Individual *Sarcocystis spp.* are named according to the accession number, definitive or intermediate host, and species of *Sarcocystis* (country). The red circle represents the *Sarcocystis spp.* identified as LC796271 in this study. rRNA, ribosomal ribonucleic acid.