c. QIAquick PCR purification kit, QIAgen, Clifton Hill, Australia.
d. pGEM-T and Wizard SV Plus Miniprep system, Promega, Annandale, Australia
e. ABI BigDye Terminator cycle sequencing mix, Applied Biosystems, Scoresby, Australia.
f. Amersham Biosciences MegaBace system, Castle Hill, Australia.
g. Sequencher™ v. 4.0.5 software, Gene Codes Corporation, Ann Arbor, MI.

References

1. Blacklock ZM, Dawson DJ, Kane DW, McEvoy D: 1983, *Mycobacterium asiaticum* as a potential pulmonary pathogen for humans. A clinical and bacteriologic review of five cases. Am Rev Respir Dis 127:214–241.
2. Cousins DV, Wilton SD, Francis BR: 1991, Use of DNA amplification for the rapid identification of *Mycobacterium bovis.* Vet Microbiol 27:187–195.
3. Dawson DJ, Blacklock ZM, Ashdown LR, Bottger EC: 1995, *Mycobacterium asiaticum* as the probable causative agent in a case of olecranon bursitis. J Clin Microbiol 33:1042–1043.
4. Ford JG, Huang AJ, Pflugfelder SC, et al.: 1998, Nontuberculosis mycobacterial keratitis in South Florida. Ophthalmology 105:1652–1658.
5. Foulkes GD, Floyd JC, Stephens JL: 1998, Flexor tenosynovitis due to *Mycobacterium asiaticum.* J Hand Surg 23:753–756.
6. Kasai H, Ezaki T, Harayama S: 2000, Differentiation of phylogenetically related slowly growing mycobacteria by their *gyrB* sequences. J Clin Microbiol 38:301–308.
7. Ladds PW: 1986, A colour atlas of lymph node pathology in cattle, pp. 10–13. P. Ladds, Townsville, Australia. [private publication ISBN 1 86252 239 1]
8. Roth A, Fischer M, Hamid M, et al.: 1998, Differentiation of phylogenetically related slowly growing mycobacteria based upon 16S–23S rRNA gene internal transcribed spacer sequences. J Clin Microbiol 36:139–147.
9. Roth A, Reischl U, Streubel A, et al.: 2000, Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S–23S rRNA gene spacer and restriction endonucleases. J Clin Microbiol 38:1094–1104.
10. Taylor LQ, Williams AJ, Santiago S: 1990, Pulmonary disease caused by *Mycobacterium asiaticum.* Tubercle 71:303–305.
11. Turenne CY, Tschetter L, Wolfe J, Kabani A: 2001, Necessity of quality-controlled 16S rRNA gene sequence databases: identifying nontuberculous Mycobacterium species. J Clin Microbiol 39:3637–3648.
12. Wayne LG, Sramek HA: 1992, Agents of newly recognised or infrequently encountered mycobacterial diseases. Clin Microbiol Rev 5:1–25.
13. Weiszfeiler JG, Karasseva V, Karczag E: 1981, *Mycobacterium simiae* and related mycobacteria. Rev Infect Dis 3:1040–1045.
14. Weiszfeiler JG, Karasseva V, Karczag E: 1971, A new mycobacterium species: *Mycobacterium asiaticum.* Acta Microbiol Acad Sci Hung 18:247–252.
15. Wilton S, Cousins DV: 1992, Detection and identification of multiple mycobacterial pathogens by DNA amplification in a single tube. PCR Methods Appl 1:269–273.
16. Wongwatana S, Sriyabhaya N: 1992, Nontuberculosis mycobacterial infection of the lung in a chest hospital in Thailand. J Med Assoc Thail 75:1–10.

J Vet Diagn Invest 17:382–385 (2005)

Natural infection of a horse with *Fascioloides magna*

S. L. McClanahan,[1] B. E. Stromberg, D. W. Hayden, G. A. Averbeck, J. H. Wilson

Abstract. A 25-year-old Quarterhorse mare was euthanized for a variety of medical reasons. At necropsy, 7 liver flukes, identified as *Fascioloides magna*, were recovered from the liver. This is the first report of *F. magna* in a horse.

Key words: Cushing's disease; *Fascioloides magna*; liver flukes; pituitary adenoma.

A 25-year-old Quarterhorse mare was presented at the Large Animal Clinic of the Veterinary Medical Center, University of Minnesota, with a 2-year history of chronic weight loss, quidding, sinusitis, and hirsutism. On admission, the mare was emaciated and had a body condition score of 1.5 (range 1.0–5.0). On the basis of physical examination, diagnoses of Cushing's syndrome, aortic regurgitation, and multiple tooth root abscesses were made. Because of its poor prognosis, the owners elected to have the mare euthanized. At necropsy, lesions unrelated to Cushing's syndrome were observed in the liver. The liver contained numerous necrotic tracts and several multifocal firm nodules (Fig. 1). The nodules were cysts that contained flukes and a dark semiliquid substance. Histopathology revealed hemorrhagic tracts and extensive areas of interstitial fibrosis. The cyst fluid and fibrotic tracts contained an abundance of fluke pigment and fluke eggs. Some eggs were free in the parenchyma, however, many were surrounded by granulomatous inflammation. Seven liver flukes ranging in size from about 3

From the Departments of Veterinary Population Medicine (McClanahan, Hayden, Wilson), Veterinary Biomedical Sciences (Stromburg), and Veterinary Pathobiology (Averbeck), College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

[1]Corresponding Author: S. L. McClanahan, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, 1365 Gortner Avenue, St. Paul, MN 55108.

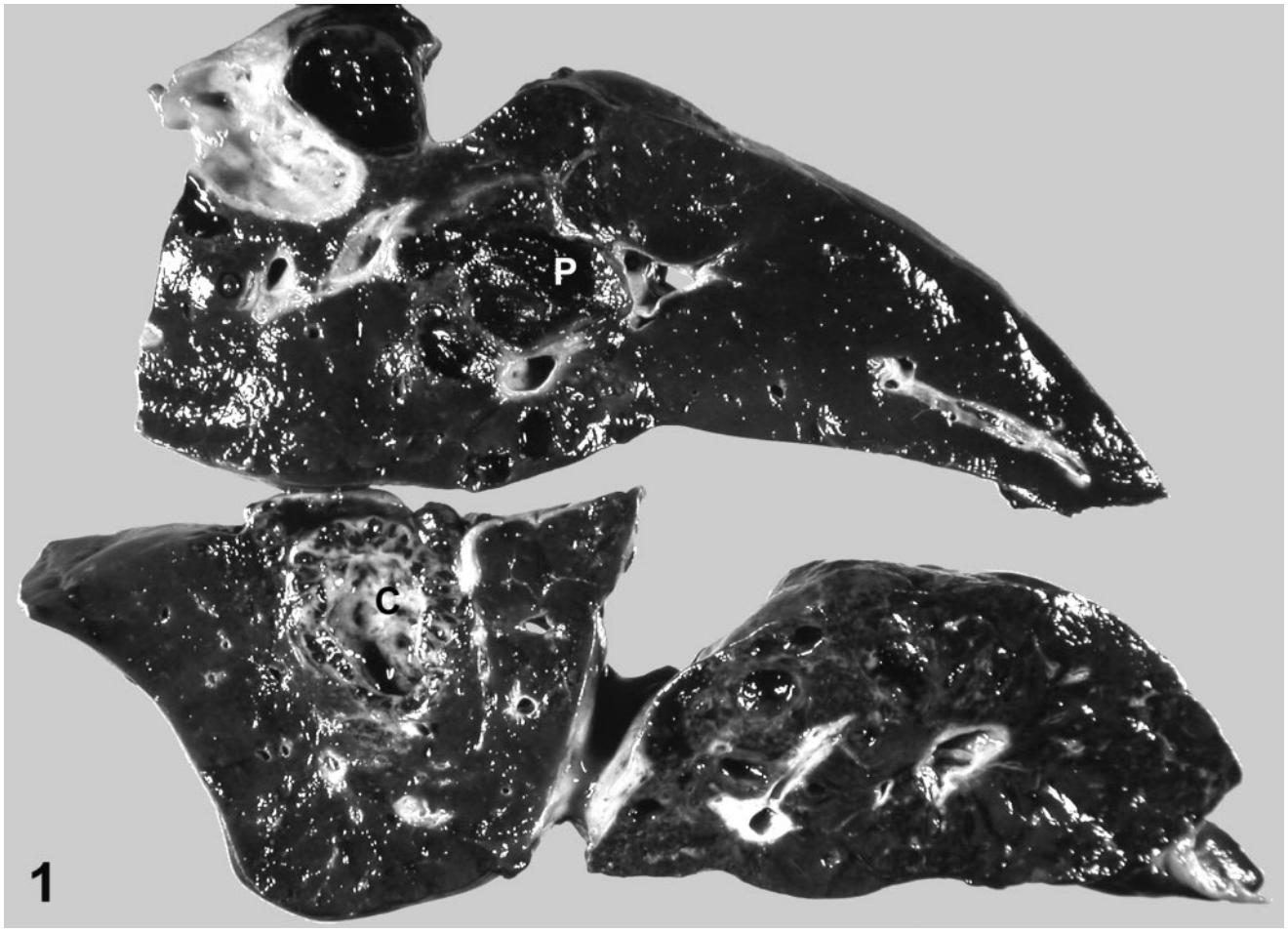

Figure 1. Sections of liver containing linear and circumscribed deposits of black pigment (P) mark the tracts of fluke migration. The lumen of a thick-walled cyst (C) that previously housed flukes is observed in 1 section.

to 8 cm were recovered from the liver (Fig. 2). The flukes were fixed in alcohol–formalin–acetic acid , and several were pressed between glass slides to facilitate subsequent staining. The smaller flukes were cleared and stained with Semichon acetic carmine. One of the larger flukes was cut in cross section, embedded in paraffin, sectioned at 5 μ, mounted on glass slides, and stained with hematoxylin and eosin (HE).

The anterior end of the fluke was rounded and lacked a cephalic cone. The ceca were highly branched. Genitalia consisted of a cirrus, uterus, ootype, a branched ovary, and 2 branched testes, which were visible in the whole-mounted flukes. In the sectioned fluke, the vitelline glands were located almost entirely ventral to the intestinal ceca. The anatomy of the flukes is consistent with *Fascioloides magna*, the large American fluke.[8] The smaller flukes were similar in size and morphology to immature flukes recovered from sheep or guinea pigs about 4 months after experimental infection with *F. magna* metacercariae.[2] The larger flukes were more similar to those recovered from naturally infected cattle or deer. Eggs were observed in the liver and in the material recovered from the cysts; however, no eggs were found in the feces using a fecal sedimentation technique.[a]

The horse had been purchased 3 years before presentation and had an unknown history. Subsequent to its purchase, the horse was grazed in a marshy area of central Minnesota that was not considered endemic for *F. magna*.[9] The horse had been routinely dewormed with ivermectin, oxibendazole, and pyrantel pamoate. The referring veterinarian had reported elevated levels of aspartate aminotransferase and gamma-glutamyl transferase.

This is the first report of *F. magna* infection in a horse. One report alluded to *F. magna* infection in the horse; however, no references substantiating this could be found in the literature.[10] The presence of small adult flukes migrating in the liver parenchyma suggests that infection had taken place within the past year. Central Minnesota has a large population of white-tailed deer (*Odocoileus virginianus*), the definitive host for *F. magna,* and there were numerous species of potential snail hosts[5] in that area. This infection would suggest

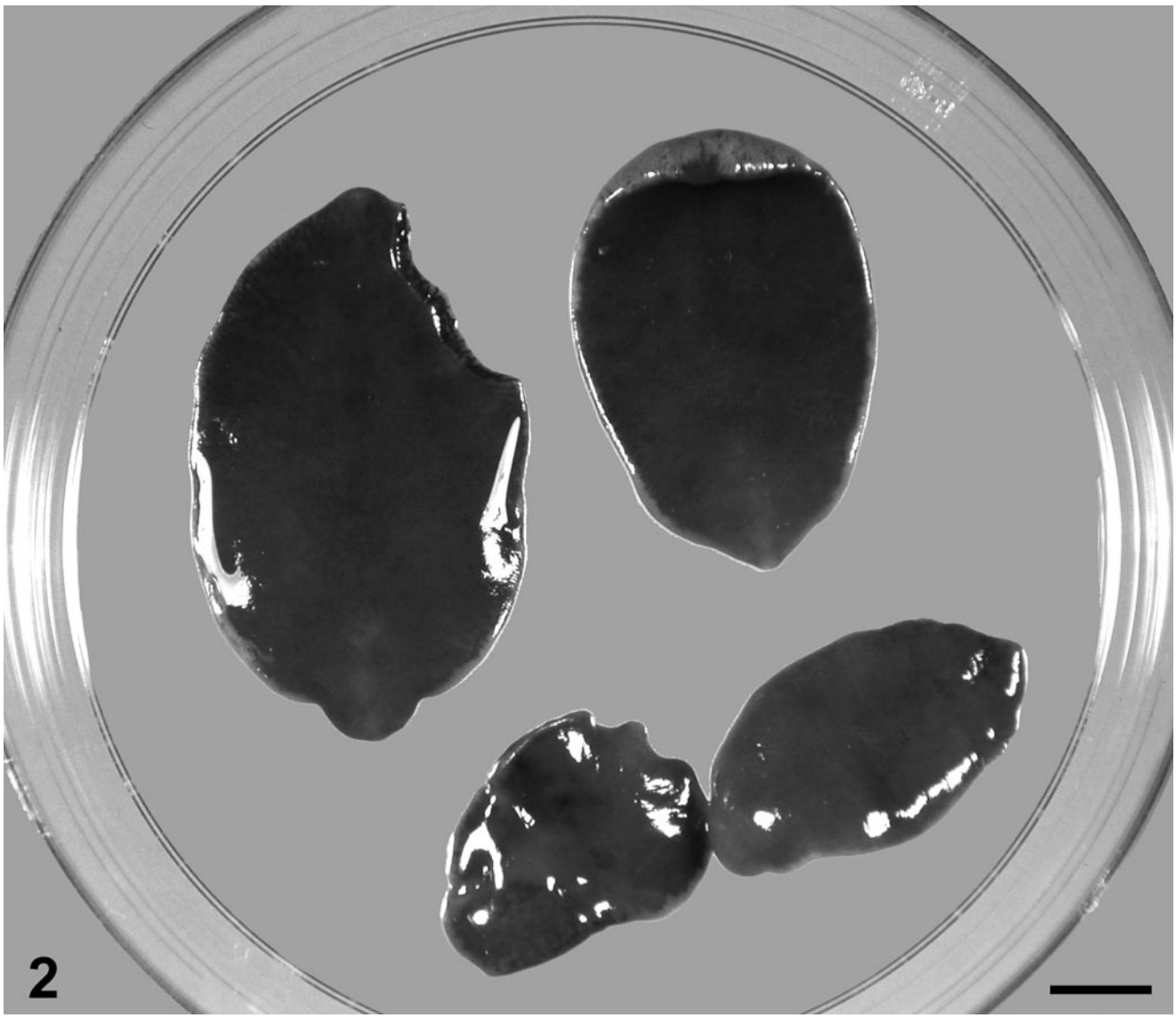

Figure 2. Four adult *Fascioloides magna* from the liver of the horse. Bar = 2 cm.

that the range of *F. magna* infection is expanding southward. The adult flukes found in cysts in the liver parenchyma were similar to those found in deer. There are few reports of *F. magna* in monogastrics. There are reports of this fluke in pigs, rabbits, mice, and guinea pigs.[2–4] However, it has been reported that the inoculation of metacercariae of *F. magna* into a Shetland pony did not result in infection.[3] It appears that this infection was more similar to that found in cattle, where encysted flukes mature and lay eggs but rarely find their way into the bile ducts and eventually feces. The closely related fluke *Fasciola hepatica* has been reported in horses throughout the world.[1,7]

Horses with Cushing's syndrome may present with chronic infections and heavy gastrointestinal parasitism, which results from cortisol-induced immunosuppression.[6] Pituitary adenoma was found at necropsy confirming that the mare had Cushing's syndrome. This may have had some effect on the flukes developing in the horse.

Acknowledgement. We thank Dr. Erik J. Olson (Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota) for the digital photography.

Sources and manufacturers

a. Flukefinder, 5051C Old Pullman Road, Moscow, ID.

References

1. Buchnell DG, Gasser RB, Beverage I: 1995, The prevalence of gastrointestinal parasites of horses in Victoria, Australia. Int J Parasitol 25:711–724.

2. Conboy GA, Stromberg BE: 1991, Hematology and clinical pathology of experimental *Fascioloides magna* infection in cattle and guinea pigs. Vet Parasitol 40:241–255.
3. Foreyt WJ: 1979, *Fascioloides magna:* development in selected nonruminant mammalian hosts. Exp Parasitol 47:292–296.
4. Krull WH: 1935, Experiments with *Fascioloides magna* involving the final host. N Am Vet 16:26–28.
5. Laursen JR, Averbeck GA, Conboy GA, Stromberg BE: 1992, Survey of pulmonate snails of central Minnesota. I. Lymnaeidae. J Freshw Ecol 7:25–33.
6. McCue PM: 2002, Equine Cushing's disease. Vet Clin N Am Equine Pract 18:533–543.
7. Owen JM: 1977, Liver fluke infection in horses and ponies. Equine Vet J 9:29–31.
8. Stiles CW: 1894, The anatomy of the large American fluke (*Fasciola magna*) and a comparison with the other species of the genus *Fasciola s.st.* J Comp Med Vet Arch 15:161–178.
9. Stromberg BE, Schlotthauer JC, Karns PD: 1983, Current status of *Fascioloides magna* in Minnesota. Minn Vet 23:8–13.
10. Swales WE: 1935, The life cycle of *Fascioloides magna* (Bassi, 1875), Ward, 1917, as a parasite of ruminants. Can J Res 12: 177–215.

J Vet Diagn Invest 17:385–388 (2005)

A study of mutations in the *c-kit* gene of 32 dogs with mastocytoma

Federica Riva, Stefano Brizzola, Damiano Stefanello, Simone Crema, Lauretta Turin[1]

Abstract. Mutations in the intracellular juxtamembrane domain of the *c-kit* gene in 32 dogs with different grades of histologically confirmed mastocytoma were studied. Transcript RNAs extracted from neoplastic tissue surgically collected from dogs of different breeds and from a negative control were reverse transcribed into complementary DNA and amplified by polymerase chain reaction. The region corresponding to the *c-kit* juxtamembrane domain was sequenced and compared with GenBank sequences. Two different types of mutations were identified within exon 11: a previously underscribed single-nucleotide substitution and a 6-bp deletion. The *c-kit* juxtamembrane domain sequences of all dogs were grouped in 3 clusters. No mutations were detected in tissues constitutively expressing *c-kit* (cerebellum and spleen), obtained from dogs not affected by mastocytoma (controls). All the substitutions were found in dogs bearing grade I or II mast cell tumors; the deletion was detected in 1 dog with grade II mastocytoma.

Key words: Canine; *c-kit*; dogs; KIT receptor; mastocytoma; mutation; oncogene.

Mast cell tumors or mastocytomas are some of the most frequently diagnosed skin neoplasms in dogs, accounting for 16–21% of all skin tumors and 11–27% of all malignant skin tumors. Most mastocytomas arise from the dermis and subcutaneous tissues; widely disseminated mastocytosis is not common (less than 10% of cutaneous cases).[7] The biological behavior of mastocytomas is highly variable; some tumors have benign behavior, whereas others have aggressive growth and metastasize.[2] A number of different parameters have been described as predictors of the clinical behavior of canine mastocytoma, the most consistent of which are histologic grade and clinical stage.[6] In recent years, molecular parameters have received more focus.

The proto-oncogene *c-kit*, encoding the transmembrane c-kit protein (KIT) receptor, is known to play a critical role in development of mast cell tumors.[5] Two mechanisms have been proposed for mast cell proliferation: ligand dependent and ligand independent.[1] One of the mechanisms proposed for the ligand-independent mast cell proliferation in dogs is the alteration of the nucleotide sequence of the *c-kit* gene within the juxtamembrane domain.[4] The aim of this study was to identify the types and locations of *c-kit* mutations in a large number of canine mastocytomas, with a view to establishing correlations between *c-kit* mutations and histological classification or clinical features, or both.[6] Moreover, the authors were interested in verifying whether such mutations could be used as a diagnostic or prognostic tumor marker for mastocytoma.

The mutational status of the *c-kit* region encompassing exons 9–13 was evaluated in a heterogeneous population of 32 spontaneous canine mastocytoma cases that were followed up for over a year. Abnormal masses were detected by clinical examination. Clinical signs of cutaneous mastocytomas included firm, elevated, circumscribed, often swollen, or ulcerated lesions of the dermis from less than 1 cm to greater than 10 cm in diameter. The area appeared to be alopecic

From the Dipartimento di Patologia Animale, Igiene e Sanita' Pubblica Veterinaria (Riva, Crema, Turin), and the Dipartimento di Scienze Cliniche Veterinarie (Stefanello), Universita' degli Studi di Milano, and the Associazione Oncologi Veterinari, Milano 20133, Italy (Brizzola).

[1]Corresponding Author: Lauretta Turin, Sezione di Microbiologia e Immunologia Veterinaria, Via Celoria 10, Milano 20133, Italy.