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Ampullary Carcinoma in a Group of Aged Rhesus Macaques (*Macaca mulatta*)

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Ampullary carcinoma was diagnosed in 6 rhesus macaques that ranged in age from 20 to 35 years. Signalment, premonitory signs of disease, and results of clinical biochemical and hematologic analyses varied among animals. Histologically, the neoplastic cells obliterated the ampulla, with regional spread to the duodenum in all 6 animals and to the pancreas in one animal. Two animals had metastases to the lung, and another two had metastases to the pancreoduodenal lymph nodes and liver. One animal had mesocolonic metastasis. Malignant tumors of the ampullary region are rare in domestic animals, and account for less than 5% of all cancers of the digestive tract in humans.

The anatomy of the ampulla of Vater in most primates, including macaques (16), is identical to the well described structure in humans (4). Formed by the junction of the common bile duct and main pancreatic duct, this short tube opens into the duodenal lumen at the apex of the major duodenal papilla. Tumors arising from this region are histologically similar to those arising from the pancreatic or bile duct and the periampullary region. Separating such tumors by origin is considered important in human cases, with ampullary carcinomas having better prognosis with increased survivability compared with that of all other periampullary carcinomas (6, 8, 9). Due to the paucity of case reports, the biological behavior of ampullary carcinomas versus cholangiocarcinomas or duodenal adenocarcinomas in other species is not known. The following cases were observed in a colony of 1,200 indoor-housed rhesus macaques over a four-year period. All animal care and experimentation in this colony is conducted entirely in accordance with relevant local, state, and national regulations, using protocols approved by the appropriate institutional Animal Care and Use committees.

Animals had been tested for Cercopithicine herpesvirus-1 (CHV-1) (antibody-based enzyme-linked immunosorbent assay), simian immunodeficiency virus (SIV), type-D retrovirus (SRV), and simian T-lymphotrophic virus [STLV-1; antibody-based enzyme immunoassays, followed by immunoblot (western blot) analysis if results were equivocal or positive] in five of six of the cases; all had been screened for reaction to mammalian old tuberculin within 12 months prior to death. Animals were provided food and water ad libitum. Five of the six animals had been fed Purina Lab Diet 5038 Monkey Diet (Richmond, Ind.), and one had received Teklad purified diet (Harlan Teklad, Madison, Wis.) for experimental purposes.

Clinical Findings

Case 1 was a 25-year-old male macaque that was born in the colony, assigned to the aging colony, and had been most recently used in breeding. Status of CHV-1 and retroviruses was unknown. Diarrhea was reported 2 weeks prior to euthanasia, and culture of a rectal specimen at that time failed to reveal known pathogens. Diarrhea continued, and dehydration developed one week after the initial complaint. Supportive care consisting of intravenously administered fluids, and a liquid diet was instituted. Physical examination (PE) 10 days after the initial report revealed a palpable mass believed to be associated with the small intestine. Exploratory surgery was scheduled, but the monkey's condition continued to deteriorate, and euthanasia was performed.

Case 2 was a 20-year-old female macaque that was born in the colony and assigned to a long-term dietary restriction study. One month prior to euthanasia, progressive microcytic anemia and low serum iron concentration were detected. A PE performed at that time did not reveal abnormalities other than obesity, and results of plain abdominal radiography were unremarkable. Two weeks later, radiographic contrast study revealed appropriate barium passage through the proximal portion of the small intestine after oral gavage, but failure to reach the ileocecal junction after 2 h. At exploratory surgery 2 weeks later, a vegetative mass was found 2 cm distal to the pylorus at the site of the ampulla of Vater, and euthanasia was performed.

Case 3 was a 22-year-old female pseudohermaphrodite that was born in the colony and was part of a group that had been exposed to androgens in utero. This animal was test positive for CHV-1 and STLV-1. Weight loss, icterus, and lethargy had been reported within two days of beginning pioglitazone treatment as part of an ACUC-approved experimental protocol. Initial clinicopathologic testing revealed increases in glucose, cholesterol, triglycerides, total bilirubin, γ-glutamyltransferase, and alkaline phosphatase values. The experimental treatment was suspended due to the onset of clinical signs of disease and suspected cholestasis. Serum biochemical analysis 2 weeks later revealed improvement in the aforementioned values, but they had not normalized. A PE was performed, and results indicated an enlarged but not turgid liver, and ultrasonography revealed mild dilatation of the biliary tree. Exploratory laparotomy performed 2 weeks later revealed a nodule in the duodenal lumen, and severe dilatation of the common bile duct. Euthanasia was performed.

Case 4 was a 35-year-old, wild-caught female macaque as-

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Table 1. Clinical signs of disease in the macaques of this report

	Cases					
	1	2	3	4	5	6
Age (yr)	25	20	22	35	25	26
Sex	M	F	Ps	F	MC	M
History	CB	CB	CB	WC	CB	B at HPL*
CS/conditions						
Diarrhea	X					
Palpable mass	X				X	X
Anemia	X	X	X	X	X	X
Weight loss	X	X		X	X	X
Icterus			X			
Lethargy			X	X	X	X
Enlarged liver			X	X	X	
Inappetence			X	X		X
Vomiting				X		X

*Born at the Harlow Primate Laboratory.
M = Male; F = female; Ps = pseudohermaphrodite; MC = male castrate; CB = colony born; WC = wild caught; CS = clinical signs of disease; and X = condition present.

signed to the aging colony. It was test positive for STLV-1. Inappetence had developed 5 weeks prior to euthanasia. Five days after the initial report, this animal was found down in the cage with irregular breathing. Clinicopathologic testing revealed mild respiratory alkalosis with metabolic compensation and hypokalemia. Also noted was increased rectal temperature of 40°C. Treatment was instituted with 360 ml of lactated Ringers solution given subcutaneously on day 1, acetaminophen (50 mg, p.o.) given once or twice daily for 5 days, and penicillin (450,000 U, i.m.) given for 7 days. The fever responded to empiric treatment. Subsequent serum biochemical analysis (performed 2, 9, and 13 days after initial assessment) revealed moderate increases in liver enzyme activities and bilirubin concentration that decreased over time. Inappetence and weight loss failed to resolve, and the animal was found down in the cage again 2 weeks later with rectal temperature of 40.5°C. Euthanasia was performed. Of note, this animal was the dam of case 3.

Case 5 was a 25-year-old castrated male macaque that was born in the colony and was not assigned to any projects at the time of death. This animal shared the same sire with case 1. It tested positive for STLV-1. The initial report was of weight loss; palpation at that time revealed soft masses in the distal portion of the abdomen. Weight loss progressed rapidly after initial presentation (12% in 2 weeks); the animal became increasingly lethargic and weak. Euthanasia was performed.

Case 6 was a 26-year-old male macaque that was born at the Harlow Primate Laboratory and assigned to the aging colony. It tested positive for CHV-1. This animal presented initially with inappetence and vomiting. Results of PE at that time indicated a mid-abdominal mass (size not specified). Serum biochemical analysis performed at the initial PE indicated acute processes that resolved quickly compared with values obtained a week later that included moderate increase in bilirubin and blood urea nitrogen concentrations and electrolyte imbalances. The latter two conditions were attributed to the vomiting, and the bilirubin increase to possible hepatic insult. Other signs of disease were not reported for three and a half months when persistent inappetence and weight loss had developed. After 5 months in all, weight loss exceeded of 20%, and exploratory laparotomy was performed. The monkey was euthanized without recovery after an intestinal mass with presumptive metastases to the liver was identified. A synopsis of notable clinical findings is summarized in Table 1.

Table 2. Necropsy findings for the affected macaques

	Cases					
Diagnosis	1	2	3	4	5	6
Ampullary CA	X	X	X	X	X	X
Metastasis-liver	X					X
Metastasis-lymph node	X					X
Metastasis-mesocolon						X
Metastasis-lung		X				X
Cholangiohepatitis	X		X	X	X	
Endometriosis		X				
Islet amyloidosis			X		X	
Uterine leiomyoma			X	X		
Granulomatous steatitis				X	X	
Gastritis					X	X

CA = carcinoma.

Necropsy Results

Relevant necropsy results for all 6 cases are summarized in Table 2. The gross appearance of the neoplasm was similar in all cases. Four of the cases (No. 2-5) had corrugation and thickening of the periampullary mucosal surface, with variable amounts of erythema or ulceration. The ampullary opening in these cases varied from indiscernible to severely widened. The remaining 2 cases actually had discrete mass lesions associated with the ampullary orifice (Fig. 1).

Four of the macaques (No. 1, 3, 4, and 5) had moderate to severe cholangiohepatitis, and *Escherichia coli* was isolated in pure culture from swab specimens of the bile duct material and liver abscesses from two (No. 3 and 5, respectively). Appreciable gross or microscopic hepatic pathologic changes were not seen in cases 2 and 6, despite multifocal hepatic metastases in case 6. Other lesions were not considered related to the ampullary carcinoma, and are found with variable frequency in rhesus macaques from this colony.

The ampullary carcinomas also were histologically similar (Fig. 2). Generally, the normal lamina propria and mucosa were replaced and expanded by a neoplastic proliferation of epithelial cells. The neoplastic cells frequently lined variably sized papilliferous projections of fibrovascular stroma and often formed acini or glandular structures. Cells were columnar, with basally oriented nuclei and moderate amounts of apical, eosinophilic cytoplasm. Nuclei were oval, with moderately clumped chromatin and zero to two round, basophilic nucleoli. Mitotic figures varied from 1 per 10 40× fields to 5 per 1 40× field. Acinar structures occasionally were dilated and filled with basophilic granular material and cell debris. Multifocally, the submucosa often contained acini and islands of the neoplastic cells that were surrounded by proliferations of fibroblasts (desmoplasia). In these foci, the cells had increasingly basophilic cytoplasm, a tendency to pile up, and the larger number of mitotic figures. In some areas, the lamina propria had been lost, and was replaced by the neoplastic cells and proliferating fibroblasts. In these type of foci, the cells rarely formed acini and had a sheet-like arrangement, but did have an appearance and mitotic rate similar to those that invaded the submucosa. In the metastatic foci, the neoplastic cells looked similar to those found in the ampullary region, but foci often had a necrotic central region.

Relevant biochemical and hematologic results are given in Table 3. All animals had mild to moderate anemia, with mild to moderate decrease in mean corpuscular volume. All animals also had low or low-normal albumin values. Other changes in parameters lacked consistency among the cases.

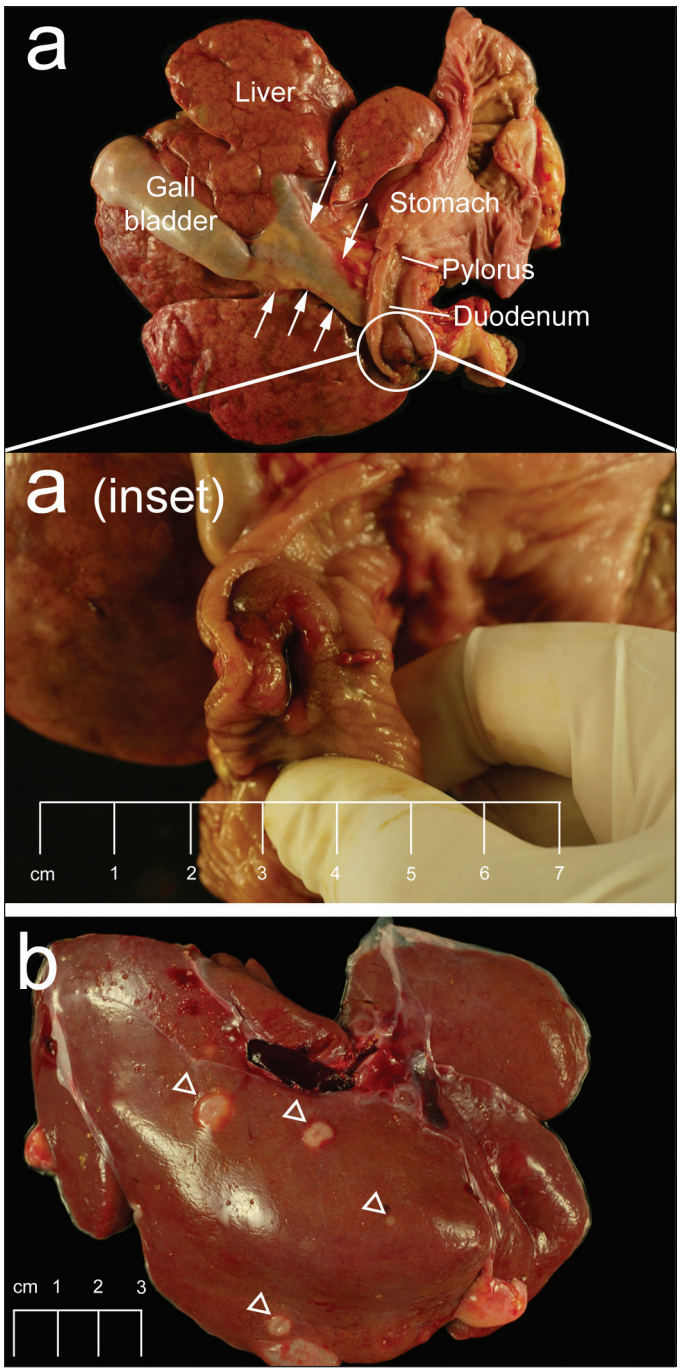

Figure 1. Gross photographs of (a) liver, stomach, and duodenum from case 4, and (b) liver from case 6. In (a), solid arrows point to the distended bile duct connected to the ampulla of Vater, which lies within the encircled mass lesion in the duodenum. The inset of (a) further magnifies the mass lesion, in which the greatly enlarged orifice of the ampulla is evident in the center of the tumor. In (b), the open arrows point to hepatic metastases of the ampullary carcinoma. The surrounding parenchyma was grossly and microscopically within normal limits.

Discussion

Ampullary carcinoma was diagnosed in 6 macaques over a four-year period from a population with a stable census of between 1,100 and 1,200 animals. This incidence is remarkable in that ampullary carcinoma had not been previously diagnosed in macaques in this colony, nor has it been reported elsewhere in the literature to our knowledge.

The question of possible influence of genetic factors is difficult to ascertain, but is hard to dismiss. The six affected animals originated from only four family lines. Two of the affected monkeys (No. 1 and 5) had the same sire, who was lost to follow-up when it was sold in 1982. Of the other 12 offspring of this male, all were lost to follow-up due to perinatal death or terminal experiments, or removal from the colony by sale. Case 3 is the result of an experiment where case 4 was treated with an androgenic steroid while pregnant with case 3. Case 4 was wild caught, and therefore, information is not available about its parentage. Of the three other offspring of case 4, two were lost to follow-up when sold, and the third remains alive and healthy in this colony at 22 years of age. Case 2 was unrelated to any of the other affected monkeys.

Four of the six affected animals were born in the colony (No. 1, 2, 3, and 5), and case 6 originated from the Harlow Primate Laboratory. Although they were not known to be related, many of the animals from Harlow share the same lineages as those housed at the Primate Center and, due to the frequently fluid division between these facilities at times in their shared history, it is impossible to say with certainty that case 6 was not related to other animals in this investigation.

Concerning etiopathogenesis, published literature relating to human ampullary carcinomas makes frequent, vague references to carcinogens in the bile or pancreatic secretions playing a role in pathogenesis of this tumor (7, 8). To our knowledge, work defining the nature of these carcinogens or their suggested mode of action is not available. References comparing bile in nonhuman primates versus humans are scarce, but in gall bladder bile in baboons, contrasted with that in humans, potassium, calcium, and total bilirubin concentrations are higher in the latter (11). Cholyglycine concentration was markedly higher in baboons. Viscosity, pH, cholesterol, sodium, chloride, bicarbonate, and phosphorus values were not significantly different between the two species. At this time, it seems reasonable to assume that differences in bile composition would not account for the increased frequency in this macaque colony versus human populations.

On a cellular level, the most common immunohistochemical stain applied to tissue sections from human ampullary carcinoma cases is for one of the cell cycle controllers, a mutant variety of p53 (12, 13, 17). Positivity of ampullary carcinomas for p53 varied from 11 to 71% in the studies reviewed. The most comprehensive immunohistochemical evaluation of this tumor investigated not only p53 expression, but also p21WAF1/cIP1, p27Kip1, p16INK4, cyclin E, and pRb, with accompanying Ki-67 antigen analysis (17). Expression of at least two of these cell cycle regulators was changed in all 14 tumors stained, suggesting that deregulation of G1/S transition is a common event in ampullary carcinoma in humans.

A definite link has been established between human patients with familial adenomatous polyposis (FAP) and carcinomas of the periampullary and ampullary regions. Due to the common practice of colectomy, periampullary carcinoma is now the leading cause of death in patients with FAP (9, 18). Evidence is also mounting that periampullary and ampullary carcinomas in FAP and non-FAP patients are associated with adenomatous lesions that progress to carcinoma (2, 9, 15). A necropsy record of an adenomatous lesion of the ampulla or the periampullary region

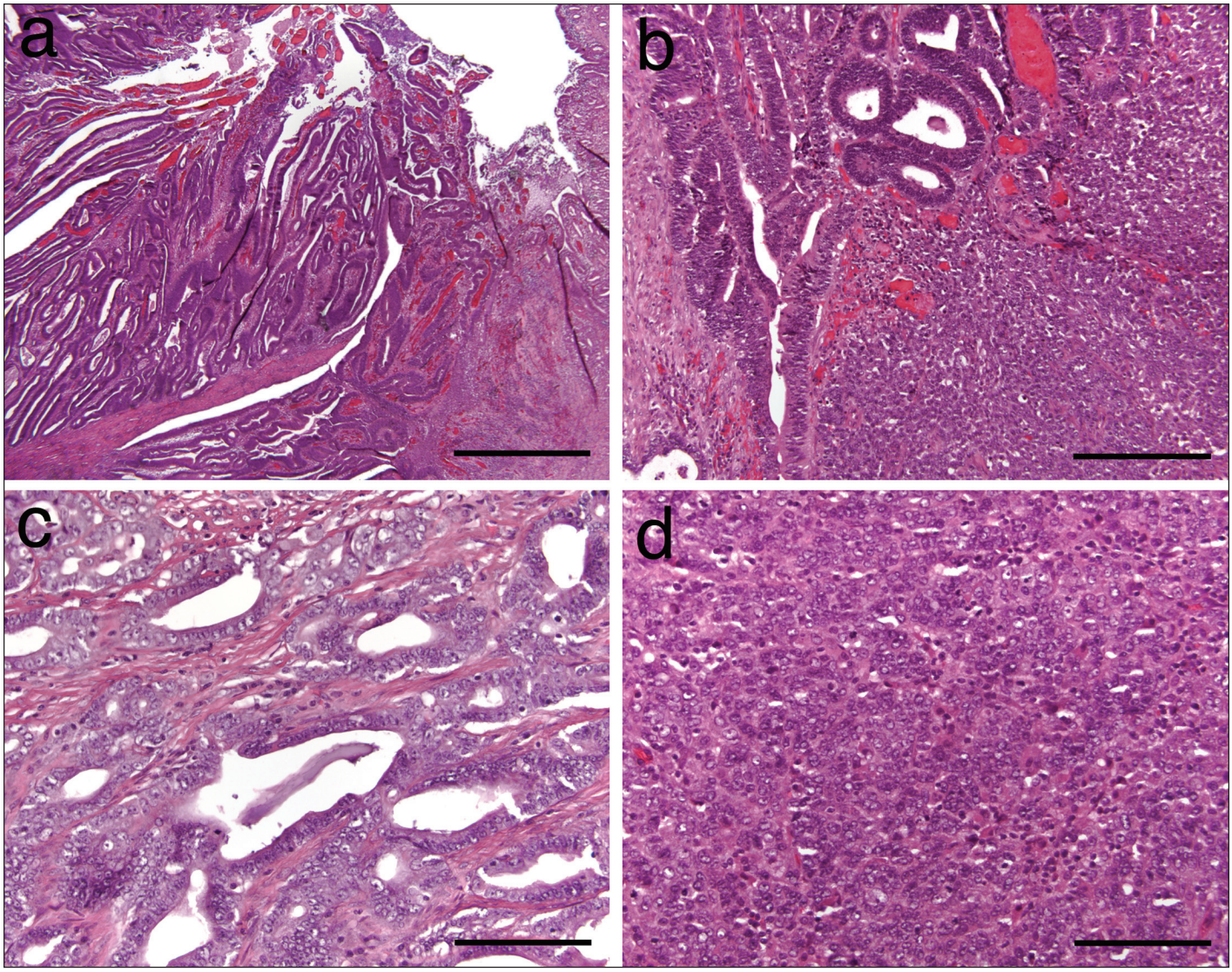

Figure 2. Photomicrographs of hematoxylin and eosin-stained sections from the ampullary carcinomas of case 4 (a) and case 2 (b-d). In (a), the normal lamina propria is replaced by papilliferous projections of stroma covered by columnar epithelial cells (bar = 1 mm). In (b), the two configurations of the neoplastic cells are evident, with cells arranged in acini on the right and in sheets on the left (bar = 200 µm). Higher magnification of neoplastic epithelial cells arranged in variably sized acini (c) also demonstrates the surrounding desmoplastic stroma, while the cells forming sheets (d) have scant stroma but similar nuclear characteristics and mitotic rates as do the acinar cells (bars = 100 µm).

Table 3. Clinicopathologic test results for the affected macaques

	Cases						Colony normal values	
Analyte	1	2	3	4	5	6	Mean	SD
BUN (mg/dl)	38	12	11	17	27	48	19	5
CK (mU/ml)	1,015	313	57	29	38	140	188	30
Chol. (mg/dl)	47	116	237	396	139	128	145	26
AST (mU/ml)	107	30	18	48	38	43	36	8
T bili. (mg/dl)	0.4	< 0.1	0.5	1	0.4	0.1	0.2	0.4
GGT (mU/ml)	65	38	240	108	47	51	46.5	13
Alb (g/dl)	2.3	2.3	2.5	1.6	2.1	3.3	3.7	0.4
AP (mU/ml)	72	541	1,391	1,059	742	146	130	41.5
Ca (mg/dl)	7.6	8.6	8.7	6.5	8	9.7	9.7	0.5
Fe (µg/dl)	186	18	69	46	74	151	117	29.5
K (mmol/L)	2.5	4.3	4.9	3.9	4.2	3.3	4.3	0.4
Cl (mmol/dl)	104	109	108	99	112	105	109	3
Hct (%)	31.2	33.3	36.2	32.9	35.1	32.1	41.5	3.6
WBC ($\times 10^3$/µl)	10.1	16.2	6.6	7.3	5.6	5.4	8.5	2.3
MCV (fl)	69.9	50.4	65.2	62	64	72	76.6	3

BUN = blood urea nitrogen; CK = creatine kinase; Chol = cholesterol; AST = aspartate transaminase; T bili = total bilirubin; GGT = γ-glutamyltransferase; Alb = albumin; AP = alkaline phosphatase; Hct= hematocrit; WBC = white blood cells; and MCV = mean cell volume.

was not found in the electronic database of this macaque colony, which dates back further than 20 years.

Infective agents have not been definitively associated with ampullary carcinoma in humans. Affected macaques from this colony did not have a consistent pattern of infection with any of the viruses tested, including CHV-1, SRV, STLV, or SIV. Agents associated with human gastrointestinal neoplasia such as *Helicobacter* spp. or Epstein Barr virus have not been found in aging macaques from this colony.

In the affected macaques from this colony, weight loss and lethargy were the most common presenting signs of disease, with the former being found in approximately 80% of the cases and the latter being present in 66% of the cases. These nonspecific signs of disease are typical for many conditions in this stoic species, who can deteriorate significantly before caretakers notice premonitory signs. In humans, the most frequent presenting signs and symptoms are jaundice and abdominal pain, both being reported in > 50% of patients (8). In macaques, presence of abdominal pain is difficult to judge, but may be a cause of the lethargy and inappetence associated with this disease. In some cases, as the disease progressed, the ampullary opening was destroyed, increasing the diameter of the passage from the common bile duct to the duodenum and allowing retrograde passage of ingesta and bacteria. In these instances, obstructive jaundice would not be expected. In the one reported case in a cat (5), the presenting sign of disease also was jaundice. The presence of microcytic anemia in affected animals is also considered a nonspecific sign of chronic disease. Case reports in humans have associated ampullary carcinoma with the Leser-Trélat sign (the sudden appearance and rapid increase in size and number of multiple sebborheic keratoses [10]) and aplastic anemia associated with androgenic steroid therapy (3).

Treatment of macaques from this colony that have been diagnosed with ampullary carcinoma is not frequently attempted. This is due to several factors, including decreased usefulness of the monkey as an experimental animal, possible need for intensive, long-term maintenance care by the veterinary and animal care staff, and frequent presence of metastases by the time the primary tumor is diagnosed. In an animal in which pancreaticoduodenectomy was attempted, postoperative hemorrhage was the cause of death. In humans undergoing pancreaticoduodenectomy, the most common postoperative complication is infection, and the overall five-year survival is approximately 50% (1, 14).

In conclusion, the reason for the cluster of ampullary carcinoma in this colony is unknown. Possible factors influencing the number of cases may relate to colony aging, genetics, more thorough necropsies, or presence of yet unidentified carcinogens that affect regulation of the cell cycle. Further investigation should center on reporting and investigation of cases from other aging colonies of rhesus macaques and immunohistochemical analysis of cell cycle regulators on tissue sections from existing cases.

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References

1. **Allema, J. H., M. E. Reinders, T. M. van Gulik, D. J. van Leeuwen, P. C. M. Verbeek, L. T. de Wit, and D. J. Gouma.** 1995. Results of pancreaticoduodenectomy for ampullary carcinoma and analysis of prognostic factors for survival. Surgery **117:**247-253.
2. **Björk, J., H. Åkerbrant, L. Iselius, A. Bergman, Y. Engwall, J. Wahlström, T. Martinsson, M. Nordling, and R. Hultcrantz.** 2001. Periampullary adenomas and adenocarcinomas in familial adenomatous polyposis: cumulative risks and APC gene mutations. Gastroenterology **121:**1127-1135.
3. **Fujino, Y., Y. Ku, Y. Suzuki, T. Ajiki, Y. Hasegawa, and Y. Kuroda.** 2001. Ampullary carcinoma developing after androgenic steroid therapy for aplastic anemia: report of a case. Surgery **129:**501-503.
4. **Gray, H.** 2000. Anatomy of the human body. Bartleby.com. [Online]. http://www.bartleby.com/107/. Accessed July 2003.
5. **Haines, V. L., P. R. Brown, R. H. Hruban, and D. L. Huso.** 1996. Adenocarcinoma of the hepatopancreatic ampulla in a domestic cat. Vet. Pathol. **33:**439-441.
6. **Howe, J. R., D. S. Klimstra, R. D. Moccia, K. C. Conlon, and M. F. Brennan.** 1998. Factors predictive of survival in ampullary carcinoma. Ann. Surg. **228:**87-94.
7. **Jean, M. and K. Dua.** 2003. Tumors of the ampulla of Vater. Curr. Gastroenterol. Rep. **5:**171-175.
8. **Jeppsson, B. and S. Bengmark.** 1995. Tumors of the papilla and ampulla, p. 2788-2791. *In* W. S. Haubrich, F. Schaffner, and J. E. Berk (ed.), Bockus gastroenterology, vol. 3, 5th ed. W. B. Saunders Co., Philadelphia.
9. **Kim, M-H., S-K. Lee, D-W. Seo, S. Y. Won, S. S. Lee, and Y-II. Min.** 2001. Tumors of the major duodenal papilla. Gastrointest. Endosc. **54:**609-620.
10. **Klimopoulos, S., C. Kounoudes, C. Pantelidaki, K. Skrepetou, M. Papoudos, and H. Katsoulis.** 2001. The Leser-Trélat sign in association with carcinoma of the ampulla of Vater. Am. J. Gastroenterol. **96:**1623-1626.
11. **Kobayashi, T., S. Taniguchi, Y. Ye, M. Niekrasz, B. Nour, and D. K. C. Cooper.** 1998. Comparison of bile chemistry between humans, baboons, and pigs: implications for clinical and experimental liver xenotransplantation. Lab. Anim. Sci. **48:**197-200.
12. **Lee, C. S. and A. Pirdas.** 1995. p53 protein immunoreactivity in cancers of the gallbladder, extrahepatic bile ducts and ampulla of Vater. Pathology **27:**117-120.
13. **Li, X., A-M. Hui, Y-Z. Shi, L. Sun, T. Takayama, and M. Makuuchi.** 2002. Deregulation of G1/S transition is a common event in carcinoma of the ampulla of Vater. Hepatogastroenterology **49:**1239-1244.
14. **Roberts, R. H., J. E. J. Krige, P. C. Bornman, and J. Terblanche.** 1999. Pancreaticoduodenectomy for ampullary carcinoma. Am. Surg. **65:**1043-1048.
15. **Seidel, G., M. Zahurak, C. Iacobuzio-Donahue, T. A. Sohn, N. V. Adsay, C. J. Yeo, K. D. Lillemoe, J. L. Cameron, R. H. Hruban, and R. E. Wilentz.** 2002. Almost all infiltrating colloid carcinomas of the pancreas and periampullary region arise from in situ papillary neoplasms, a study of 39 cases. Am. J. Surg. Pathol. **26(1):**56-63.
16. **Swindler, D. R.** 1973. An atlas of primate gross anatomy, p. 214. University of Washington Press, Seattle.
17. **Teh, M., A. Wee, and G. C. Raju.** 1994. An immunohistochemical study of p53 protein in gallbladder and extrahepatic bile duct/ampullary carcinomas. Cancer **74:**1542-1545.
18. **Tomita, H., H. Fukunari, M. Shibata, K. Yoshinaga, T. Iwama, and Y. Mishima.** 1996. Ampullary carcinoma in familial adenomatous polyposis: report of a case. Surg. Today **26:**522-526.