# Prevalence of and risk factors associated with atherosclerosis in psittacine birds

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SMALL ANIMALS/ AVIAN

# Prevalence of and risk factors associated with atherosclerosis in psittacine birds

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**Objective**—To estimate the prevalence of clinically relevant atherosclerotic lesions in birds and identify epidemiological variables and illness types associated with development of atherosclerosis.

**Design**—Retrospective case-control study.

**Sample**—Records of 7,683 psittacine birds, including 525 with advanced atherosclerosis.

**Procedures**—5 pathology centers provided databases and access to histopathology slides. Age and sex were collected for all birds of the *Amazona*, *Ara*, *Cacatua*, *Nymphicus*, and *Psittacus* genera. Databases were searched for atherosclerosis cases, and slides were reviewed for the presence of type IV through VI atherosclerotic lesions. Results were used to build several multiple logistic models to define the association between advanced atherosclerosis and age, sex, genus, illness type, and specific lesions. Prevalence was reported as a function of age, sex, and genus.

**Results**—In the first model including 7,683 birds, age, female sex, and the genera *Psittacus*, *Amazona*, and *Nymphicus* were significantly associated with clinically relevant atherosclerosis detected via necropsy. Subsequent models of 1,050 cases revealed further associations with reproductive disease, hepatic disease, and myocardial fibrosis, controlling for age, sex, and genus.

**Conclusions and Clinical Relevance**—Age, female sex, and 3 genera appeared to be positively associated with the presence of advanced atherosclerotic lesions in psittacine birds. This information may be useful in clinical assessment of the cardiovascular system and patient management. Reproductive diseases were the only potentially modifiable risk factor identified and could be a target for prevention in captive psittacine birds. (*J Am Vet Med Assoc* 2013;242:1696–1704)

Atherosclerosis is a chronic inflammatory fibroproliferative vascular disease characterized by the buildup of atheromatous materials composed of numerous compounds including inflammatory cells, lipid, calcium, and collagen in the luminal aspect of the arteries in response to multiple forms of endothelial injuries. Complications of atherosclerotic lesions lead to well-characterized common diseases in humans, including coronary artery disease, ischemic stroke, and peripheral arterial disease. As in humans, some species of birds seem extremely susceptible to atherosclerosis; therefore, avian species, including pigeons, quails, and chickens, have been extensively studied. Studies[1–3] on these species have elucidated some important aspects of the pathogenesis and treatment of atherosclerosis. Among birds, psittacines seem particularly prone to developing spontaneous lesions principally in the large arteries at the heart base.[4–7] However, scientific investigations are scarce and certainly not of a scale in proportion to the importance of the disease in this taxon. Most studies[4–11] have focused on prevalence and histologic lesions.

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**Abbreviations**

| | |
|---|---|
| CI | Confidence interval |
| ROC | Receiver operating characteristic curve |

The histologic lesions have been well described in psittacines and appear similar to those in humans and compatible with the classification system developed by the American Heart Association.[5,6,12,13] Psittacine atherosclerotic lesions consist of the progressive accumulation of inflammatory cells, cholesterol, fat, and cellular debris in the intima and luminal side of the media. In advanced lesions, there is also formation of a lipid core (atheroma; type IV lesion) covered by a fibrous cap (fibroatheroma; type V), and complications such as fissures, hematomas, and thrombosis (type VI) may occur.[5] In humans, clinically important lesions are defined as types IV through VI on the basis of reports from the American Heart Association Committee on Vascular Lesions.[13,14] Human postmortem and clinical imaging studies[13,15,16] reveal that humans with clinical signs of atherosclerosis have lesions of type IV or higher.[13,15,16]

The epidemiology of atherosclerotic diseases is well described in humans but, for the most part, unknown in psittacine birds. Few studies[17–22] have investigated the effect of dietary factors in psittacine birds. Comparison of the epidemiology of atherosclerosis in humans and psittacines is appropriate because they are both long-lived species and may share common risk factors. The reported prevalence for atherosclerosis in psittaciformes varies widely from 1.9% to 92.4%, depending on the study and species.[4,6–10,17,a] Postmortem retrospective studies and prospective studies on the prevalence of atherosclerosis in psittacines often have variable study designs, have different or poorly defined inclusion criteria often encompassing all lesion types, and do not control for confounding factors and covariables such as age and sex. Furthermore, there is a need to model the prevalence of such slowly developing lesions as a function of age. The prevalence of atherosclerosis in psittacine birds of various ages is unknown but appears greater in older birds.[4–6] Knowing the prevalence of advanced and clinically important atherosclerosis is crucial to the development and interpretation of diagnostic tests (with regard to positive and negative predictive values) but also constitutes an invaluable aid in assessing and managing psittacine patients on the basis of their signalment and medical history.

Risk factors are an individual's measurable characteristics that predict the probability of developing clinical disease. This definition does not imply a causal relationship but suggests that risk factors must occur before the disease.[23] Risk factors for atherosclerotic diseases in humans include lifestyle (smoking, diet, physical inactivity, stress, abuse of alcohol, and obesity), biochemical abnormalities (high concentrations of cholesterol, triglyceride, low-density lipoprotein, C-reactive protein, and lipoprotein a; thrombogenic factors; low high-density lipoprotein concentration; and homocysteinemia), physiologic abnormalities (hypertension, diabetes mellitus, and metabolic syndrome), and personal factors (age, sex, and genetics).[23,24] In birds, genetic risk factors are present in White-Carneau pigeons and in quails that are susceptible to experimental atherosclerosis.[25,26] In psittacines, predisposed species have been suggested, and age, diet, and dyslipidemia are also frequently cited as potential risk factors,[4–6] but these simple potential risk factors have not been confirmed by studies that used a large representative parrot sample and robust statistical methods.

In birds, atherosclerotic lesions can remain subclinical for an extended period until the accumulation of atheromatous materials leads to pathological complications and subsequent clinical signs caused by ischemia, arterial aneurysm, stenosis, and cardiac consequences.[9,10,27–33] Therefore, simple reports of the prevalence of lesions of atherosclerosis and their associations with several variables (that cannot be risk factors) are of modest immediate clinical interest. Considering the implications in the definition of a risk factor, we suggest that veterinary clinicians would be better served by knowing risk factors for advanced atherosclerotic lesions rather than for lesions of various severities that may remain subclinical for decades. Atherosclerosis-induced avian diseases are challenging to diagnose, and a better knowledge of their epidemiology and association with other diseases in psittacine species would undoubtedly improve birds' medical care in captivity.

The purpose of the study reported here was to determine associations between specific variables and advanced atherosclerotic lesions in 5 psittacine genera. The hypothesis was that the prevalence of atherosclerosis would increase with age and that sex and some genera would be associated with a higher prevalence than that of other factors. We also hypothesized an association with cardiac, hepatic, reproductive, and renal diseases.

## Materials and Methods

**Case-control selection and study design**—The available databases of 5 pathology centers were reviewed for all necropsy cases (excluding biopsy and cytology cases) for which the heart was evaluated for the following 5 genera of psittaciformes: *Psittacus* (African grey parrots), *Amazona* (Amazon parrots), *Cacatua* (cockatoos), *Ara* (macaws), and *Nymphicus* (cockatiels). Databases included the School of Veterinary Medicine at Louisiana State University, Baton Rouge, La (2008 through 2010); the Ontario Veterinary College at the University of Guelph, Guelph, ON, Canada (2001 through 2011); the Schubot Center at Texas A&M University, College Station, Tex (1988 through 1998); the Zoo/Exotic Pathology Service, Sacramento, Calif (1998 through 2011); and Northwest ZooPath, Monroe, Wash (1997 through 2012).

Species, age, and sex were recorded for all birds when available. Information from all submissions was reviewed to ensure they were necropsy submissions and not biopsy submissions. The words atherosclerosis and arteriosclerosis and their misspelled variants were used to search the databases for psittacine birds with a diagnosis of atherosclerosis. All the histopathology slides of these cases were retrieved and reviewed for the inclusion criteria. Only birds with advanced atherosclerotic lesions of type IV through VI (according to a described classification system in psittacines[5]) in the brachiocephalic, pulmonary, carotid, or coronary arteries or the aorta were included as cases in the study. These types of lesions are defined as the clinically important lesions that may induce clinical signs in humans.[13,14] Parrots that had other types of atherosclerotic lesions (I, II, III, or VII) or lesions in other locations were not included as cases.

All psittacine birds were included in a first logistic model assessing the association between the prevalence of atherosclerosis and age, sex, and psittacine genera. Second and third logistic models were built with birds included as atherosclerotic cases and a subset of the remaining birds included as controls, which were randomly sampled via statistical software.[b] The number of controls selected was equal to the number of cases. The criterion for control selection was limited to failure of the bird to have advanced atherosclerotic lesions as evaluated via histologic examination. Availability of patient information and completeness of pathology records were not required for selection, to prevent potential selection bias. Furthermore, to address bias associated with centers, locations, databases, and pathologists, controls were matched with cases at each institution.

A more detailed history was then recorded for all cases and controls including species, age, sex, types of histologic lesions found (inflammatory, infectious, neoplastic, degenerative, traumatic, metabolic, and other), and organ systems affected (hepatic, gastroenteric, reproductive, musculoskeletal, respiratory, neurologic, cardiovascular [other than atherosclerosis], integumentary, endocrine, lymphoid [splenic or bursal], and renal, as well as systemic lesions). Lesions interpreted as mild by the pathologists were not included in the analysis. The occurrence of specific diseases that were potentially associated or concomitant with atherosclerosis was recorded; the list of such diseases comprised *Chlamydophila psittaci* infection, potential herpesvirus infection (internal papillomatosis and Pacheco's disease), vacuolar hepatopathy, hepatic fibrosis, renal glomerulopathy, nephrosis, myocardial fibrosis, myocardial degeneration, and myocarditis.

**Statistical analysis—**Data regarding age, sex, or both were missing in 26.6%, 25.2%, and 7.3% of medical records (unknown or unreported on submission sheets), respectively, and were assumed to be missing at random. To limit the loss of information and bias associated with listwise deletion (the default category of the statistical software), the missing values were dealt with via multiple imputations.[34] A bootstrap-based expectation-maximization-Bayesian algorithm was used to perform 5 multiple imputations by missing value, creating 5 datasets.[34] Age was included in the imputation algorithm with a log transformation to achieve normality, which was 1 assumption of the method.

Results were used to first construct a multiple logistic model to predict the prevalence of advanced atherosclerosis on the basis of age, sex, and genus and to then quantify the effects of these potential risk factors. This regression model was fitted simultaneously with the combination of each of the 5 entire datasets corresponding to the 5 imputations with the presence of severe atherosclerosis as the binary outcome variable with age, sex, and genera (dummy variables) as explanatory variables. Final model parameters were combined by means of Rubin's rules either manually or automatically by the statistical software.[34,35] To compare the difference in prevalence among genera, the reference level for genera was accordingly changed in the model. To obtain the OR for each genus, a separate model with only 1 of the 5 genera dummy variables was run for each genus. To assess the validity of the imputation procedure, imputation diagnostic tests were performed by comparison of the distribution of the imputed data with the complete data and by comparison of the OR obtained via the model that had the complete-cases dataset (4,264 birds) with the model that used multiple imputations.

The median effect level was reported as the age for which a 50% probability of having advanced atherosclerosis was observed (50% prevalence), which was equal to the following:

$$(-\text{intercept} - \Sigma \text{ dummy variables parameters}) / \text{age parameter, where logit}(0.5) = 0$$

To investigate further associations, a more complex second multiple logistic model was subsequently fitted with only the cases and randomly sampled controls and with the addition of types of diseases and organ systems affected as explanatory variables. Only a few interaction terms corresponding to the specific research hypothesis were part of the models (eg, degenerative X cardiac). For this model, because of the high number of variables, a backward stepwise selection process was performed to find the best model (to exclude = 0.15; to include = 0.10). This was done manually to avoid removing variables that were part of an included interaction term. Different models were compared via the Akaike information criterion. Multicollinearity was checked with variance inflation factors prior to performance of stepwise procedures.

Finally, a third logistic model investigating the association between severe atherosclerosis and specific lesions or diseases (*C psittaci* infection, herpesvirus infection [internal papillomatosis and Pacheco's disease], vacuolar hepatopathy, hepatic fibrosis, renal glomerulopathy, nephrosis, myocardial fibrosis, myocardial degeneration, and myocarditis) was fitted, controlling for age, sex, and genus. The explanatory variables in the second and third models were not combined, as they were assumed to be multicollinear.

Assumptions of the models and the presence of outliers were checked on the basis of model fit and residual plots. Fit and predictive power of the models were assessed on the basis of the area under the ROC. Odds ratios were obtained by exponentiation of the parameter estimates and were reported with their 95% CIs. A type I error rate of 0.05 was used for determination of significance. Statistical analysis was performed with statistical software, and related programming packages were used for multiple imputations[c] and combinatory logistic models.[d]

## Results

A total of 7,683 parrots were evaluated in the study, with 525 confirmed advanced atherosclerosis cases (IV through VI), which represented an overall prevalence of 6.8% (95% CI, 6.2 to 7.4) when the model was not controlled for age, sex, and genus. The distribution of age in birds with a known age (5,640 birds) was determined (**Figure 1**). Of the parrots for which sex was known (5,750 birds), 51.5% were females and 48.5% were males. The overall population comprised 17.9% *Psittacus* spp, 14.9% *Amazona* spp, 26.4% *Nymphicus* spp, 16.1% *Cacatua* spp, and 24.7% *Ara* spp. The control-to-case ratio for the first logistic model was 13.6. There was only a mild difference in the point estimates of the ORs between results of the analysis that used complete information and the larger analysis that used multiple imputations (**Table 1**).

Age, sex, and genera had a significant association with the prevalence of advanced postmortem atherosclerotic lesions when other variables in the models were held constant (**Figures 2–4**; Table 1). Older parrots and female parrots had greater odds of developing advanced lesions. Males had 69% the odds of having advanced lesions, compared with females. Among genera, in descending order, African grey parrots, Amazon parrots, and cockatiels were particularly susceptible to atherosclerosis and had increased odds of having severe lesions, compared with the other genera in the model,

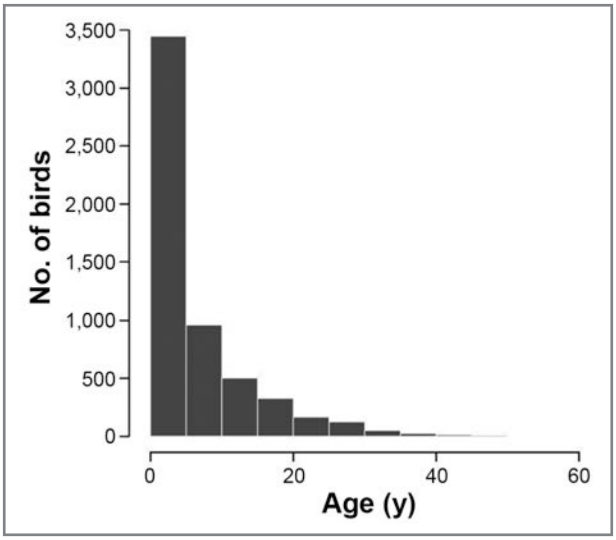


Figure 1—Distribution of ages of 5,640 psittacine birds evaluated in a study of advanced atherosclerosis for which age was known.

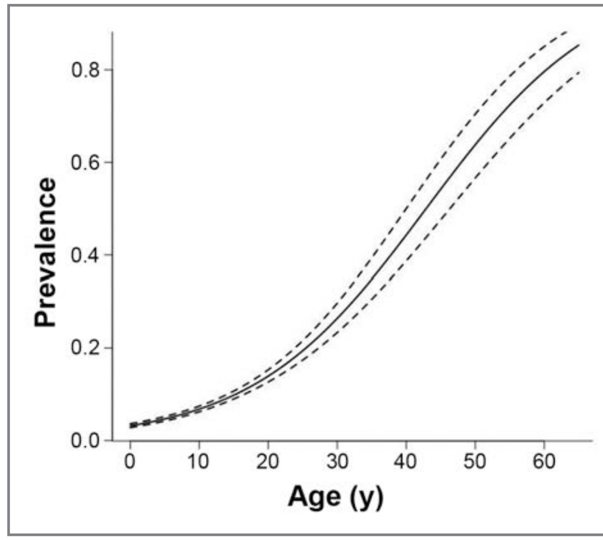


Figure 2—Estimated prevalence of advanced atherosclerotic lesions as a function of age (solid line) in a population of 7,683 psittacine birds. Dotted lines represent 95% CIs.

Table 1—Odds ratios of age, sex, and genus for the presence of advanced atherosclerosis on necropsy in 7,683 psittacine birds.

| Variable | OR | 95% CI | *P* value | ORcc |
|---|---|---|---|---|
| Age | | | | |
| 1-year increase | 1.08 | 1.07–1.10 | < 0.001 | 1.10 |
| 10-year increase | 2.22 | 1.93–2.57 | < 0.001 | 2.59 |
| Sex (male/female) | 0.69 | 0.56–0.85 | 0.007 | 0.66 |
| Genus | | | | |
| *Psittacus* | 2.75 | 2.23–3.45 | < 0.001 | 2.54 |
| *Amazona* | 1.83 | 1.45–2.30 | < 0.001 | 1.75 |
| *Nymphicus* | 1.46 | 1.14–1.87 | < 0.001 | 2.02 |
| *Cacatua* | 0.25 | 0.16–0.38 | < 0.001 | 0.20 |
| *Ara* | 0.13 | 0.08–0.20 | < 0.001 | 0.13 |

The *P* value for genera is for comparison with the overall mean response.
ORcc = OR for complete-case dataset on 4,264 birds (without use of multiple imputation procedures).

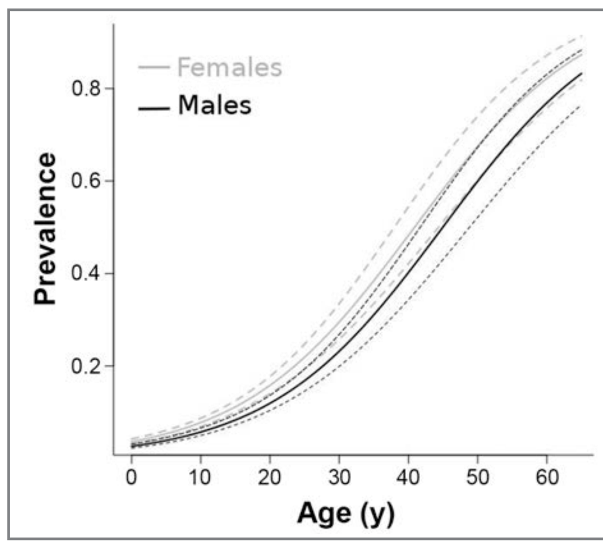


Figure 3—Estimated prevalence of advanced atherosclerotic lesions as a function of age and sex (solid lines) in a population of 7,683 psittacine birds. Dotted lines represent 95% CIs.

and had a higher prevalence than psittacines overall. Cockatoos and macaws seemed relatively less susceptible than the overall population, with odds of 25% and 13%, respectively, compared with all birds. Genera differed significantly from each other (*Psittacus-Amazona* comparison, $P = 0.017$; all others, $P < 0.01$) except for *Amazona*, which did not differ significantly in prevalence from *Nymphicus* ($P = 0.38$), and *Cacatua*, which did not differ significantly in prevalence from *Ara* ($P = 0.11$).

In males, the median age effect (50% prevalence of advanced atherosclerosis) was observed at 34.7, 40.3, 40.6, 60.5, and 63.5 years in *Psittacus*, *Amazona*, *Nymphicus*, *Cacatua*, and *Ara*, respectively. In females, the median age effect was observed at 30.8, 36.0, 36.7, 56.4, and 59.8 years in *Psittacus*, *Amazona*, *Nymphicus*, *Cacatua*, and *Ara*, respectively. The oldest cockatiel in the study was 30 years old, but the median age effect may still be a useful value to report in this species for comparison purposes.

The area under the ROC was 0.83 for these models, indicating a good fit. No outlying data were detected, and the interaction between sex and age was not significant.

The second logistic model investigating association with disease revealed that birds with reproductive or hepatic disease were at increased odds of advanced atherosclerosis on postmortem examination, controlling for age, sex, and genus (**Table 2**). The association with hepatic disease was less robust than with reproductive disease. Most birds with reproductive disease were females (49/50). Birds with cardiac degenerative disease were at increased odds, but this was not significant. The third logistic model revealed a significant increase in odds for myocardial fibrosis diagnosed along with atherosclerosis, controlling for age, sex, and genus. Only the ORs for positive parameter estimates with $P < 0.10$ of the final selected models are reported. The area under the ROC was 0.86 and 0.85 for the second and third logistic models, respectively.

Atherothrombotic lesions, aortic rupture, aortic dissection, and plaque hemorrhage (atherosclerotic lesion type VI) were rarely diagnosed, appearing in only

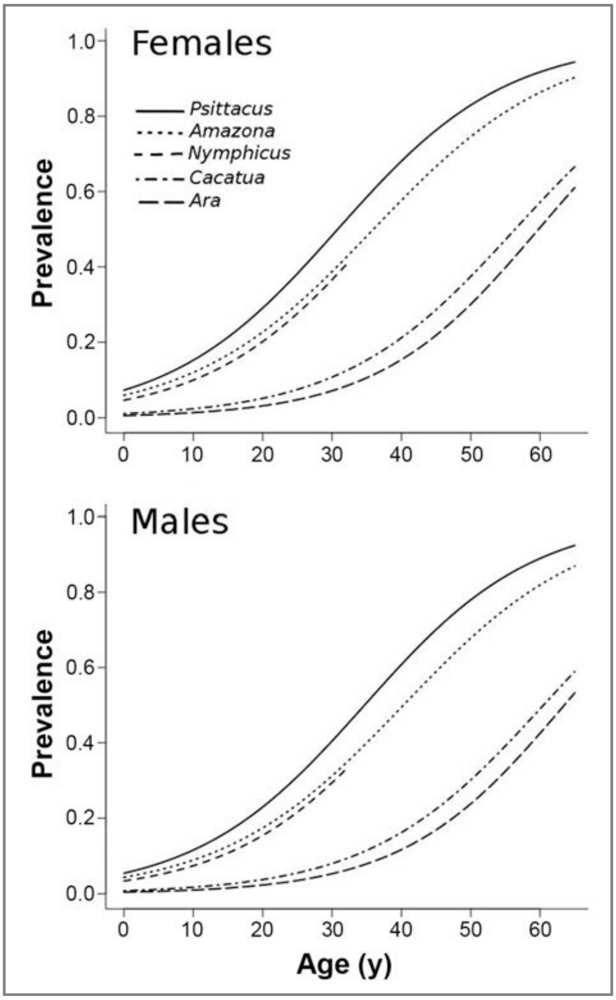


Figure 4—Estimated prevalence of advanced atherosclerotic lesions as a function of age, sex, and genus in a population of 7,683 psittacine birds.

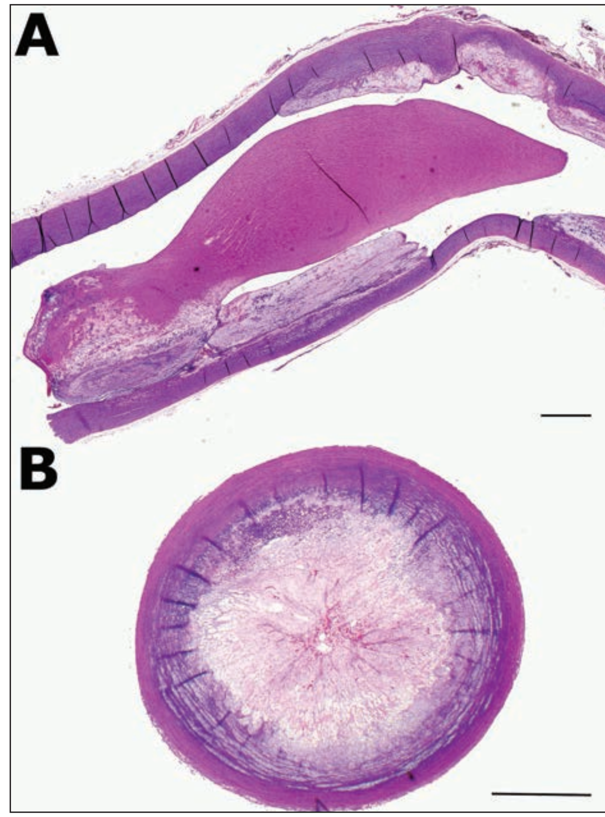


Figure 5—Photomicrographs of atherosclerotic lesions in parrots. A—Atherosclerotic lesion type VI in a longitudinal section of the aorta of a 13-year-old female African grey parrot. The lesion is composed of a large eroded atheromatous plaque in the intima, connected to an organized fibrin thrombus that partially obstructs the lumen. H&E stain; bar = 1 mm. B—Atherosclerotic lesion type IV in a cross section of the brachiocephalic artery of an Amazon parrot. The lesion is composed of a large circumferential atheromatous plaque that nearly completely obstructs the arterial lumen. No fibrous cap overlying the lesion is evident. H&E stain; bar = 1 mm.

Table 2—Odds ratio for presence of disease and lesion association with the presence of advanced atherosclerosis at necropsy in psittacine birds in case-control logistic models with 525 cases, controlling for age, sex, and genus.

| Variable | OR | 95% CI | *P* value |
|---|---|---|---|
| Second logistic model | | | |
| Reproductive diseases | 3.09 | 1.39–6.88 | 0.007 |
| Hepatic diseases | 1.58 | 1.05–2.39 | 0.030 |
| Degenerative cardiac diseases | 2.62 | 0.90–6.95 | 0.080 |
| Third logistic model | | | |
| Myocardial fibrosis | 4.68 | 1.83–12.00 | 0.001 |

Only variables with positive parameter estimates and $P < 0.10$ are reported.

1.9% (10/525) of cases but always considered to have contributed to the death of the bird, on the basis of pathologists' comments (**Figure 5**). Although difficult to objectively assess because of postmortem arterial collapse and contraction, severe stenosis sometimes with near complete occlusion seemed a fairly common finding with advanced lesion types and always contributed to the death of the bird, according to the pathologists. Only 1 bird with advanced atherosclerosis had a concomitant diagnosis of *C psittaci* infection.

## Discussion

This observational retrospective study determined the prevalence and association of advanced lesions of atherosclerosis and selected epidemiological factors and concurrent diseases in a large sample of parrots from 5 centers and for 5 of the most common genera evaluated by avian practitioners. Results indicated, through multiple logistic modeling, that age, female sex, and *Psittacus*, *Nymphicus*, and *Amazona* genera were associated with increased odds of developing clinically important atherosclerotic lesions. The large sample size permitted estimation of the prevalence by age, sex, and genus with high precision. Atheromatous lesions were also significantly and positively associated with reproductive disease and mildly associated with hepatic disease and concurrent myocardial fibrosis. One can assert that the inclusion criteria for the cases were too stringent, leaving some types of atherosclerotic lesions in the control population, or that the association between advanced lesion types and clinical signs remains to be proven in psittacine birds. Although we concur with these arguments, several factors supported the validity of the study design. The advanced lesions (types IV through

VI) are qualified as clinically important in the American Heart Association classification scheme and include lesions that induce clinical signs and subclinical lesions that have not yet caused disease by vascular occlusion or thromboemboli.[13,14] This lesion classification derived from human pathology seems to be supported in birds on the basis of the present study findings (stenosis was common) and a number of case reports[9,10,27,29–31] in which clinical signs could clearly be associated with arterial luminal narrowing and ensuing tissue hypoperfusion but not with atherothrombosis, plaque rupture, or preatheromatous lesions (types I through III). Complicated lesions (type VI) appeared rare in parrots in the present study (1.9% of advanced lesions and 0.1% overall raw prevalence), which was consistent with previous reports.[4] However, clinical signs are not always caused by atherothrombosis, and in fact, humans have a high rate of lesions (up to 26% in 1 study[36]) associated with sudden coronary death for which thrombosis or other causes of death cannot be found on autopsy. In these cases, symptoms and deaths are suspected to be caused by arterial stenosis leading to chronic myocardial ischemia and lethal arrhythmia.[36] Furthermore, atherosclerotic diseases are challenging to diagnose in birds, and a diagnosis is often, if not always, made on postmortem examination.[4,37] Thus, in absence of a better alternative, studying the prevalence of type IV–VI atherosclerotic lesions as a surrogate for estimating the prevalence of atherosclerotic disease seems an acceptable strategy for parrots.

Results of the present study must be interpreted in the context of the study limitations. The retrospective nature of the study was associated with several drawbacks, including incomplete information, difficulties in extraction of valuable information from large databases, inability to determine causal relationships, susceptibility to bias, and assessment of risk factors after the outcome of interest had occurred.[38] The prevalence and severity of lesions found on necropsy may have also been different from the true prevalence of the live population, and advanced atherosclerotic lesions may have still been present but not represented in the available histologic sections. Sophisticated methods to deal with missing values such as multiple imputations reduce bias and increase efficiency, compared with, for instance, performing statistical analysis on only complete data or imputing the overall mean; imputations incorporate the uncertainty associated with the missing values and limit information loss.[34,39,40] In addition, all organs were not always assessed histologically, which may have introduced some bias and limited the validity of assessment of the association of general and specific lesions of other organ systems with advanced atherosclerosis. Control selection and sampling are important sources of bias, and proper methods are crucial to interpret study results.[38] For this reason, age, sex, and genus risk factors were investigated in the entire available population in the first logistic model. Control samples were collected from this population for practical reasons to investigate more specific associations and for examination of new hypotheses to be confirmed by future observations. Nevertheless, case-control studies are easier to perform than cohort studies, are inexpensive, can be performed in a short time period, and may reveal important findings.[38] The lengthy development of atherosclerotic lesions in long-lived species does not easily lend itself to cohort studies, and case-control studies are an efficient alternative for such diseases with a long latency period.[38]

Only 5 psittacine genera were included in this study, and the results may not apply to other psittacine species. There are approximately 84 genera, including 353 species, in the order Psittaciformes, and it would have been challenging to account for all of them in a balanced and meaningful statistical model, especially because some species are kept in captivity in low numbers. For that reason, it was decided to focus on only the genera most commonly evaluated by avian practitioners and pathologists.

The prevalence of advanced atherosclerotic lesions in psittacine birds was comparable to that of humans in North America, keeping in mind the longer life span of humans, with, for instance, a 30% to 50% prevalence in 45- to 75-year-old humans.[41]

Age was found to be a significant and important risk factor for advanced atherosclerosis in the studied psittacine genera. This risk increased dramatically in birds 20 to 30 years of age. Although this effect was detected in previous psittacine studies, the present study provided a more precise estimation of magnitude and a wider range of ages. A recent small case-control study[42] revealed a significant effect of age in preliminary analysis that failed to be significant in a multivariable model, but this may have been caused by the small sample size. Interestingly, the OR for age, obtained on a smaller sample, was nearly identical to that of the present study (1.08/y vs 1.09/y).[42] Age is also an important predictor of atherosclerotic diseases in humans.[24,41] The effect of age is explained by the increased exposure time to risk factors, the slow accumulation of atheromatous materials in the arteries over time, the physiologic effects of aging, and the increasing prevalence and severity of specific cardiovascular risk factors with age, such as dyslipidemias and hypertension.[24] The number of older psittacine birds evaluated by avian veterinarians is expected to increase with the improvement of conditions in captivity and advances in avian medicine, so the number of parrots with disease processes linked to atherosclerosis will likely increase.

Another important finding was the significant effect of female sex on the probability of finding advanced lesions at necropsy. A female predisposition was detected in 2 previous studies on the basis of raw percentages.[8,43] However, this effect was not detected in most previous studies,[4,6,9,17,44] probably because of low sample size and statistical power. In 1 report with > 1,000 parrots, prevalence in males was 4 times that in females, but the dataset was skewed toward male sex, and the analysis was not controlled for confounding variables.[44] Interestingly, Japanese quails that are susceptible to experimental diet-induced atherosclerosis have greater prevalence in males, but this may not be comparable to development of spontaneous lesions.[45] In the present study, males with a similar prevalence as females were approximately 4 years older. This is a reversal from mammals and humans, in which males < 55 years old have 2 to 3 times as many coronary heart disease events, strokes, or instances of peripheral arterial disease as females and have a 50% increased lifetime risk of developing coronary artery disease.[24,41] This sex difference diminishes after menopause, but the risk in women lags by 10 to 15 years behind that of men.[24,41] This effect is not completely understood but is thought to be related to sex hormones and environmental and lifestyle factors that vary between sexes.[25,46] The protective action of estrogens benefits cardiac and vascular tissues, and estrogens may also have beneficial effects on blood pressure, hemostasis, inflammation, and lipoprotein metabolism.[46,47] In fact, these effects could well be reversed in female birds, for which estrogens have tremendous physiologic effects on lipid, protein, and calcium metabolism related to reproduction and egg formation.[3,48] In reproductively active females, estrogen induces increases in plasma total calcium, protein, cholesterol, and triglyceride concentrations and hepatic synthesis of 2 specific lipoproteins, vitellogenin and very low-density lipoprotein (yolk labeled), which target the developing oocyte and are protected against degradation by plasma lipoprotein lipase.[48,49] Increased plasma cholesterol, very low-density lipoprotein, very low-density–lipoprotein remnants, and non–high-density lipoprotein cholesterol concentrations promote atherogenesis, providing a plausible explanation for the enhanced predisposition found in female psittacine birds.[24,50] Moreover, dietary supplementation of estrogens exacerbates lipid deposition in the aorta of 5-day-old female chicks.[51]

The genera found to have a predisposition in the present study provide confirmation of previously suspected or confirmed species susceptibility.[4,6,17,42] The reasons for this are unknown, but one can speculate that these genera may have physiologic and genetic differences or different captive lifestyle and stress levels that could influence atherogenesis. Also, these species have evolved with dietary and lifestyle habits that have changed drastically in captivity.[52]

In the model investigating organ system and types of diseases, the greatest effect was observed for reproductive disease, which further supported the finding of a female sex effect. These findings may reflect a more general and frequent dysregulation of the reproductive system in captive psittacine birds, compared with wild birds, which may subsequently promote chronic diseases in other organ systems.[53,54] Only a mild association was found with hepatic disease, and no association was found with vacuolar hepatopathy or hepatic fibrosis when the model was controlled for other variables. These lesions were prevalent in both cases and controls. Human patients with hepatic lipidosis are at increased risk of cardiovascular diseases through a variety of mechanisms.[55,56] Therefore, our findings may be explained by differing pathophysiology of hepatic lipidosis in psittacine species than in humans, the multiplicity of causes, an incorrect disease classification, or some unidentified bias affecting these variables such as incomplete histologic evaluation of all organs. Also, only moderate to severe lesions were included in the study. A previous study[42] failed to find any association between atherosclerosis and illness types, but some psittacine cases were concurrently reported[29,31] with hepatic lipidosis and fibrosis.

Contrary to a recent case-control study[42] that used immunohistochemical staining of arteries from 62 psittacine birds and in accordance with another study[11] that used PCR analysis and immunohistochemical staining in 103 birds, we did not find any association between avian chlamydiosis and atherosclerosis; only 1 of 525 birds was confirmed at necropsy to be infected. However, we did not attempt to detect *C psittaci* antigens in arteries. Association with *Chlamydia pneumoniae* infection in humans remains controversial after decades of research.[57–60]

Myocardial fibrosis was significantly related to the presence of advanced atherosclerotic lesions, suggesting that these vascular lesions may induce pathological cardiac changes that could lead to myocardial dysfunction, cardiac arrhythmia, or further degenerative changes. Myocardial fibrosis is a frequent finding in humans with coronary artery disease, is caused by chronic myocardial ischemia and systemic hypertension, and can lead to congestive heart failure and sudden death.[61–63] Severe myocardial fibrosis was reported in several case reports[30,31] of birds with advanced atherosclerotic lesions and in a high percentage of Amazon and African grey parrots in a previous study.[6]

Several major risk factors could not be assessed retrospectively. The diet, which is probably one of the most important modifiable risk factors, could not be investigated because it was rarely reported on submission sheets or the information was too scant to be of use. Clinicopathologic variables could not be investigated. Total cholesterol concentration was substantially higher in parrots with atherosclerosis in a previous retrospective study.[42] Total cholesterol and low-density lipoprotein concentration increase in response to 1% to 2% cholesterol feeding and experimental induction of atherosclerosis in Quaker parrots (*Myiopsitta monachus*), budgerigars (*Melopsittacus undulatus*), and other avian species.[1,10,42,64] Moreover, Amazon and African grey parrots have higher plasma concentrations of cholesterol, and the latter have increased plasma cholesterol concentrations when fed high-fat diet in feed trials.[4,19,21]

The present large multicenter study provided avian veterinarians with useful information on prevalence and risk factors for advanced lesions of atherosclerosis in captive psittacine birds, and this information may be of value in cardiovascular disease assessment and patient management. Reproductive diseases were the only potentially modifiable risk factor that was identified. There is no prospective information to definitely confirm this association; we suspect that reproductive diseases are likely to be true risk factors on the basis of the 3 following elements: presence of a significant female susceptibility, significant association found with reproductive diseases, and the common occurrence of chronic reproductive disorders in captive psittacines. Therefore, prevention and management of reproduction dysfunction in captivity and salpingohysterectomy in females may have some benefit in lowering the prevalence of atherosclerosis in psittacine birds.

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