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Risk Factors Associated with Developmental Limb Abnormalities in Captive Whooping Cranes

Cristin Kelley
International Crane Foundation

Barry K. Hartup
International Crane Foundation

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CRISTIN KELLEY, International Crane Foundation, E-11376 Shady Lane Road, Baraboo, WI 53913, USA
BARRY K. HARTUP, International Crane Foundation, E-11376 Shady Lane Road, Baraboo, WI 53913, USA

Abstract: This retrospective survey identifies potential risk factors for developmental limb abnormalities in whooping crane (*Grus americana*) chicks reared at the International Crane Foundation between 1990 and 2006. We summarized a series of biologically-relevant pre- and post-hatch variables from this population using in-house aviculture and veterinary records, and then compared them between chicks with and without developmental carpal, toe and leg deformities. Chi-square analysis revealed associations between 1) rearing style and each type of deformity, 2) egg source and carpal and toe deformities, and 3) the pre-existence of a toe deformity and carpal and leg deformities. Multivariate analyses using logistic regression suggest that 1) increased risk of toe and leg deformities were highly associated with hand rearing, 2) lower relative weight change in week 1 increased the risk of toe deformities, 3) higher relative weight change in week 2 increased the risk of leg deformities, 4) female chicks and chicks from third clutch eggs were at increased risk of leg deformities, and 5) increased risk of carpal deformities was observed in chicks from eggs collected at Wood Buffalo National Park and in chicks with pre-existing or concurrent toe deformities. This information can be used by both aviculture and veterinary staff when making decisions regarding placement, management, and veterinary care to minimize the likelihood and impact of developmental limb abnormalities in captive reared whooping crane chicks.

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Developmental limb abnormalities are of particular concern in the captive-rearing of whooping cranes (*Grus americana*). Significant resources are often committed to correcting these problems, which may result in euthanasia or withdrawal of cranes from reintroduction projects. Lateral rotation of the distal end of the carpometacarpus, rotational and linear toe abnormalities, and long bone rotational and angular pelvic limb deformities have been described in cranes (Olsen and Langenberg 1996). In this paper, we will refer to these conditions as carpal, toe, and leg deformities, respectively.

Similar abnormalities have been described in bustards (Naldo and Bailey 2001), poultry (Julian 1984, McNamee et al. 1998, Leeson and Summers 1988, Bradshaw et al. 2002), ratites (Hahulski et al. 1999), parrots (Harcourt-Brown 2004), raptors (Zsivanovits et al. 2006), flamingos (Zollinger et al. 2005), and waterfowl (Smith 1997). Several factors have been studied and/or suggested as risks for developing limb abnormalities. These include: genetics, growth rate, nutrition and/or protein type, exercise, handling, housing, rearing style, and infection (Leeson and Summers 1988, Curro et al. 1996, McNamee et al. 1998, Hahulski et al. 1999, Kirkwood 2000, Naldo and Bailey 2001, Bradshaw et al. 2002, Harcourt-Brown 2004, Zsivanovits et al. 2006).

The purpose of this study was to identify potential risk factors for developmental limb abnormalities in whooping crane chicks reared at the International Crane Foundation (ICF). This preliminary analysis may be of use for controlled research into determining causal relationships between these factors and abnormal limb development.

METHODS

The study population consisted of 179 whooping crane chicks between hatching and 70 days reared at ICF between 1990 and 2006 following ICF rearing protocols (Wellington et al. 1996). In-house records generated by ICF aviculture and veterinary staff were reviewed, and selected biological data was recovered for each individual. Information regarding the presence or absence of carpal, toe, and leg deformities was recorded. Guidelines for diagnosis of these deformities were taken from Olsen and Langenberg (1996).

Information was also recovered and organized for the following biological values for each chick: clutch (first clutch = first or second egg in sequence, and so on), egg source (collected from captive-held female = 131 [ICF = 126, USGS Patuxent Wildlife Research Center = 3, Calgary Zoo = 2] or wild nest from Wood Buffalo National Park [WBNP], Canada = 49), assisted hatch (yes/no), gender (male/female), rearing method (hand reared [includes costume reared] vs. parent reared), fledge status (survived to day 70 or not; 144 chicks in this survey fledged), fresh egg weight (g), hatch weight (g), weights at weekly intervals from hatch to day 70 (g, 7 ± 1 day intervals). Chicks that died on the day of hatch were not included in the analysis. All eggs taken from WBNP nests were assumed to be first clutch eggs based on synchrony of embryo development. Relative weight change for the preceding week, expressed on a percent basis, was calculated for all surviving chicks using the following formula: [(current weight – weight from prior week)/weight from prior week] x 100.

Comparisons were made between all variables and chicks with and without deformities. Categorical variables were evaluated using chi-square tests, and continuous variables

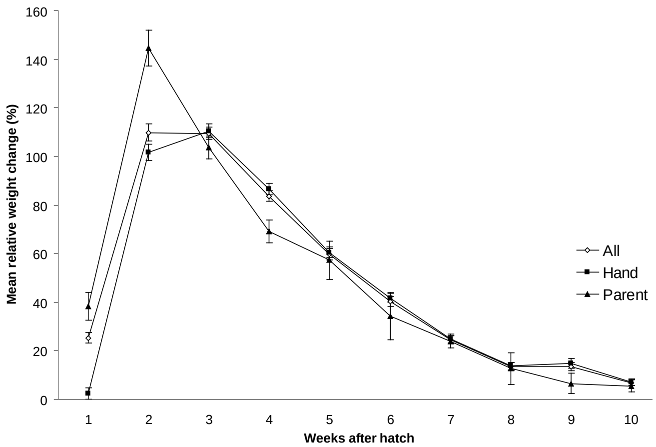

Figure 1. Mean weekly relative weight changes of all whooping crane chicks by rearing type at ICF, 1990-2006.

were evaluated using unpaired t tests. Significance was established at $P \leq 0.05$. Stepwise logistic regression analysis was then used to evaluate the potential predictive association of the study variables with the presence of each type of developmental limb abnormality (Hosmer and Lemeshow 1989). Interactions among study variables were excluded from the logistic model due to difficulty in their biological interpretation. This analysis allowed for examination of an odds ratio (OR) for each variable, adjusting for effects of all other variables remaining in the model, after stepwise removal of non-significant factors (the manual removal limit was $P > 0.15$). Significance was established at $P \leq 0.05$. The OR denotes the observed likelihood of the outcome when an individual has been exposed to a particular factor. An OR greater than one suggests increased risk or likelihood of the outcome (e.g., OR = 2 suggests individuals exposed to factor X were twice as likely to experience the outcome Y); OR less than one reduced risk or likelihood of the outcome. Statistical analyses were conducted using software for microcomputers (SAS Institute 1998, CDC 2005).

RESULTS

We identified 31 (18%) captive-reared chicks with carpal deformity, 86 (49%) with toe deformity, and 51 (29%) with leg deformity during this survey. Overall summary statistics for each variable studied are provided in Table 1. The mean fresh egg weight (± SD) for all chicks surveyed was 209.1 ± 17.3g. The mean hatch weight for all chicks surveyed was 128.7 ± 12.9g. The mean hatch weight of chicks from WBNP eggs was 10% larger than the mean hatch weight of chicks from captive eggs ($t_1 = 7.27$, $P < 0.001$).

Mean relative weight changes for all chicks, hand reared, and parent reared chicks are shown in Figure 1. Significant differences in relative weight change between hand reared chicks and parent reared chicks were found in weeks 1, 2, and 4. In week 1, parent reared chicks showed 42% greater relative weight gain than hand reared chicks ($t_1 = 2.96$, $P = 0.004$). In week 2, parent reared chicks had a 30% greater relative weight gain than hand reared chicks ($t_1 = 5.14$, $P < 0.001$). In week 4, however, hand reared chicks' relative weight gains were 20% greater than parent reared chicks ($t = 3.01$, $P = 0.003$).

The relationships of carpal, leg and toe deformity with the categorical variables are shown in Table 2. Cases of carpal and toe deformity were significantly more common in chicks from WBNP than in chicks from captive sources ($\chi^2_1 = 9.65$, $P = 0.002$ and $\chi^2_1 = 5.01$, $P = 0.025$ respectively). There was no statistical difference in the prevalence of these problems, however, between 1990 and 1996 when chicks from both sources were being raised simultaneously at ICF. Rearing style was significantly associated with all three developmental limb

Table 1. Frequency distribution of categorical variables in 179 whooping crane chicks reared at ICF, 1990–2006.

		n (%)
Clutch		
	First	92 (51)
	Second	54 (30)
	Third	33 (18)
Source		
	Captive	130 (73)
	Wood Buffalo	49 (27)
Assisted hatch		
	No	156 (87)
	Yes	23 (13)
Gender		
	Male	85 (49)
	Female	89 (51)
Rearing		
	Iso/Hand	139 (79)
	Parent	38 (21)
Fledged		
	No	36 (20)
	Yes	143 (80)
Carpal deformity		
	Yes	31 (18)
	No	145 (82)
Toe deformity		
	Yes	86 (49)
	No	90 (51)
Leg deformity		
	Yes	51 (29)
	No	125 (71)

Table 2. Analysis of associations between potential risk factors and limb deformities in whooping crane chicks reared at ICF, 1990–2006).

		Carpal deformity			Toe deformity			Leg deformity		
		Yes	No	χ^2, *P*	Yes	No	χ^2, *P*	Yes	No	χ^2, *P*
Clutch										
	First	19	70		44	45		24	65	
	Second	7	47	NS [a]	23	31	NS	14	40	NS
	Third	5	28		19	14		13	20	
Source										
	Captive	16	114	9.65, 0.002	57	73	5.01, 0.025	39	91	NS
	Wood Buffalo	15	31		29	17		12	34	
Assisted Hatch										
	No	26	127	NS	76	77	NS	43	110	NS
	Yes	5	18		10	13		8	15	
Gender										
	Female	14	75	NS	47	42	NS	32	57	NS
	Male	17	65		38	44		19	63	
Rearing										
	Iso/Hand	30	106	7.66, 0.006	85	51	42.59, <0.001	47	89	8.28, 0.004
	Parent	1	37		1	37		4	34	
Fledge										
	No	1	32	5.95, 0.014	14	19	NS	6	27	NS
	Yes	30	113		72	71		45	98	
Developmental Toe Deformity										
	No	9	81	7.36, 0.007	n/a [b]	n/a	n/a	20	70	4.08, 0.043
	Yes	22	64		n/a	n/a		31	55	

[a] Non-significant (p > 0.05)
[b] Not applicable

abnormalities. In each case, hand reared chicks were more likely to develop limb or toe deformities than chicks that were parent reared (carpal $\chi^2_1 = 7.66$, p = 0.006, toe $\chi^2_1 = 42.59$, $P < 0.001$, leg $\chi^2_1 = 8.28$, $P = 0.004$). There also was a significant association between the presence of a toe deformity and the presence of both carpal and leg deformities ($\chi^2_1 = 7.36$, $P = 0.007$, and $\chi^2_1 = 4.08$, $P = 0.043$, respectively). Female chicks tended to develop leg deformities compared to male chicks ($\chi^2_1 = 3.33$, $P = 0.068$).

Odds ratios for variables from the stepwise logistic regression models using all available chick records were generally consistent with the bivariate results (Table 3). Hand rearing was a primary risk factor for the development of toe deformities (95% confidence OR = 6.8 – 500.0) in captive reared whooping cranes at ICF, while relative weight gain during the first week was associated with a slight reduction in the likelihood of toe deformity (OR = 0.97 – 0.99). Each % increase in relative weight gain during this week corresponded with a 2% decrease in risk of toe deformity. The likelihood of carpal deformities in the chicks was increased in the presence of a toe deformity (OR = 1.1 – 6.3) or when chicks originated as a WBNP egg (OR = 1.3 – 6.8). Hand rearing (OR = 1.8 – 27), coming from an egg of a third clutch (OR = 1.1 – 8.7), being female (OR = 1.1 – 5.0), and greater relative weight gain during the second week after hatching (OR = 1.01 – 1.02) were all potential risk factors for leg deformities in the chicks during the study.

DISCUSSION

Developmental limb abnormalities in whooping crane chicks have challenged both veterinary staff and aviculturists since ICF began captive rearing of whooping cranes in 1990. While much has been published that addresses how to manage cases of developmental limb abnormalities, little beyond speculation exists regarding risk factors for these abnormalities. Our results show that hand rearing, relative weight change, concurrent toe deformities, gender (female) and clutch number (third) are potential risk factors for developmental limb abnormalities.

Known differences in parent rearing vs. hand rearing may be the key to reducing developmental limb abnormalities. Amount of exercise as well as type and timing of exercise are different between the two groups. Parent reared chicks are often on the move as they follow their parents. Hand reared chicks are housed singly in pens most of the time and rely on their human caretakers for directed exercise. At ICF, both parent and hand reared chicks have access to a high quality, balanced pelleted food specifically designed for cranes; yet, adult cranes often supplement the diets of their chicks with insects and other plant and animal matter found in the pens. In addition, hand reared chicks are subjected to physical restraint by caretakers more often than parent reared chicks due to differences in their management (Wellington et al. 1996). Handling puts stress on chicks' limbs that could lead to injuries and ultimately to developmental limb abnormalities. Future studies should be designed to quantify aspects of parent rearing in captivity to determine the behavioral, nutritional, physiological, and management factors most influential to limb development.

Table 3. Results of logistic regression models for the effects of variables on three different developmental limb abnormalities in captive reared whooping crane chicks.

Deformity / Variable	Coefficient	SE	OR[a]	95%CI[b]
Carpal				
Source (WBNP)	1.10	0.42	3.00	1.31 – 6.85
Toe deformity	0.99	0.44	2.68	1.13 – 6.34
Constant	-2.49	0.38	—	—
Toe				
Hand rearing	3.98	1.06	52.6	6.76 – 500.0
Weight change wk1[d]	-0.17	0.01	0.98	0.97 – 0.99
Egg Clutch				
Second	0.13	0.45	NS[c]	NS
Third	1.16	0.64	NS	NS
Constant	0.81	0.30	—	—
Leg				
Hand rearing	1.96	0.69	7.09	1.84 – 27.03
Egg Clutch				
Second	-0.32	0.44	NS	NS
Third	1.11	0.54	3.03	1.06 – 8.71
Sex – Female	0.84	0.39	2.32	1.07 – 4.98
Weight change wk2[d]	0.01	0.01	1.01	1.01 – 1.02
Constant	-1.58	0.62	—	—

[a]Adjusted odd ratio.
[b]Confidence interval.
[c]Not significant ($p > 0.05$).
[d]Relative (%)

Hand rearing has also been attributed to increased incidence of developmental limb abnormalities in parrots (Harcourt-Brown 2004) and raptors (Zsivanovits et al. 2006), and offers contrast to our expectations in cranes. In parrots, increased exercise in hand reared chicks may influence deformities. Parent reared parrot chicks are housed in nest boxes with their siblings, for several days or weeks depending on the species, and move very little due to the small size of the nest box and the presence of their siblings against them. Hand reared parrots are often housed singly in plastic tubs for ease of cleaning, and removed from the tubs and allowed to stand or even walk around during feedings. Hand reared raptors may also receive excessive exercise, and in addition may be being fed meats in higher quantity and quality than their parent reared counterparts. Although the exercise concerns in parrots and raptors are opposite from those of cranes, the difficulties in mimicking the exercise regimen of parent reared birds in hand reared birds are consistent across species.

Increased growth rate has been suggested as a risk factor for developmental limb abnormalities for several species. Decreased growth rate in poultry reduces the incidence of developmental limb abnormalities, and feed restriction, lighting regimes, exercise, and nutrition contribute to growth rate changes (Bradshaw et al. 2002). Ad libitum feeding, access to protein or energy rich foods and lack of exercise produce an increase in the incidence of limb abnormalities, especially among large, long-legged birds (Kirkwood 2000). In flamingos, management of leg deformity includes walking and hydrotherapy and reduction in daily weight gains by feed restriction (Zollinger et al. 2005). Our results suggest relative weight gain is a risk factor for leg deformity, specifically during the second week following hatch. Weight and relative weight change is monitored closely in chicks reared at ICF. Relative weight gains exceeding 10-15%/day are considered to be a risk for the development of leg deformities in hand reared chicks. When gains of this magnitude are detected, changes in management follow. The chicks are either fed less or exercised more, or both (Wellington et al. 1996).

Weekly weight gains have been recorded for several crane species, including Siberian *(G. leucogeranus)*, sandhill *(G. canadensis)*, sarus *(G. antigone),* brolga *(G. rubicundus),* white-naped *(G. vipio),* and red-crowned *(G. japonensis)* cranes, using data from individuals without leg deformities (Wellington et al. 1996). Hand reared whooping crane chicks showed less relative weight gain in the first week than any other species of crane (limiting the protective effects of relative weight change on toe deformities based on our results). By the second week, hand reared chick weight gains were similar to other species, and the % change is consistent with management goals that limit weight gains to ≤15%/day.

The data are striking for gains exhibited in parent reared chicks, which exceeded all other species of crane by the second week. These results indicate that parent reared whooping crane chicks, which rarely exhibit leg deformities, commonly exceed weight gains of 15% per day in the second week of development; a trend typically avoided or actively managed against in hand reared chicks. The risk (as determined by the logistic regression model) is apparently balanced by the large preventive effect of parent rearing, which we presume to be related to exercise quality that is not replicated in hand rearing situations.

Chicks resulting from eggs of third clutches (fifth and sixth sequence eggs) and female chicks may be at risk for leg deformities. We do not believe that third clutch chicks are nutritionally disadvantaged (hatch weight was not significantly different compared to second clutch hatchlings). Third clutch eggs may also have a higher probability of longer periods of artificial incubation, since these eggs arrive later in the season when incubating cranes at ICF cannot effectively incubate additional eggs naturally. Unfortunately, our study did not consider incubation parameters as potential risk factors for limb deformities because incubation is not consistent among eggs at ICF and does not lend itself to statistical analysis. Each egg receives a customized incubation regimen based on numerous factors such as available natural incubators of several species, weather, number of eggs being managed at any given time, etc. Third clutch chicks are also likely to be concurrently managed with several chicks in the hand rearing environment, which may have behavioral or physiological consequences (e.g., stress) that impact the development of leg deformities. Likewise, females may also be affected by social group dynamics; weights of ICF female chicks were not significantly different than males until 7 weeks of age (data not shown), well after the median onset of leg deformities (approximately 20 days of age). More analysis is required to discern the impact of gender on leg deformities, such as skeletal development and morphology.

We found that the presence of a toe deformity is associated with both carpal and leg deformities, though logistic regression modeling did not support the latter relationship. We suspect that chicks with toe deformities may not inherently exercise as much, may alter their food intake (particularly during the first week after hatch), and are likely handled more than chicks without toe deformities for examinations and treatment which could impact activity levels. All of these factors may lead to carpal or leg problems and require further study. This finding encourages the rapid application of treatment to quickly resolve toe deformities, in order to avoid the potential negative consequences of chronic treatment. Chicks with toe deformities should be monitored closely for leg and carpal deformities.

The risk factors of hand rearing, relative weight gains, the presence of toe deformities and the inherent factors of gender and egg sequence (clutch) should be considered in regards to the overall management of whooping crane chicks. We recommend that parent rearing should be considered whenever possible and/or feasible for optimal growth and development of whooping crane chicks. Unfortunately, whooping crane recovery efforts demand high numbers of chicks which outnumber the availability of parents to rear them, and no effective way has been developed to introduce migratory parent reared birds to the wild. Therefore, every effort should be made to adapt hand reared chick management to emulate parent rearing in a captive environment, and to manage factors such as weight and toe deformities as closely as possible. Additional studies are needed of the dynamics of parent rearing in captivity.

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Laguna de Babicora, Chihuahua, Mexico, 7,000 ft elevation, the most important sandhill wintering area in Mexico, wintering up to 50,000 cranes. Photo by Roderick C. Drewien.