

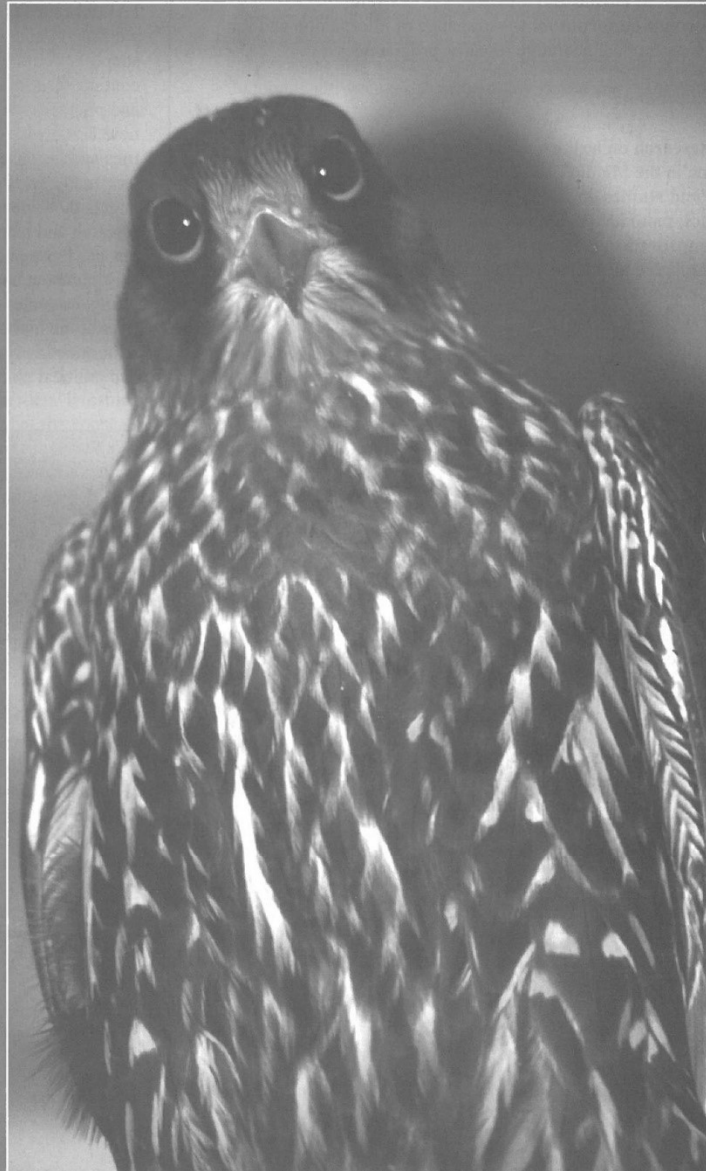
# FALCO

The Newsletter of the Middle East Falcon Research Group  
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FALCO is published biannually and contains papers, reports, letters and announcements submitted by Middle East Falcon Research Group Members. Contributions are not refereed: although every effort is made to ensure information contained within FALCO is correct, the editors cannot be held responsible for the accuracy of contributions. Opinions expressed within are those of the individual authors and not necessarily shared by the editors.

## MEFRG Objectives:

### To provide:

A **central body** for the co-ordination of research activities related to falcons and falconry.

A **common forum** for the exchange of information and for promoting collaborative research programmes.

### To promote:

**Research** on health and disease in falcons, falcon moulting in the Middle East, falcon nutrition, domestic breeding.

**Field studies** on falcon migration, taxonomy, morphometrics, reproductive biology and behaviour.

**Improved management conditions** for captive falcons through educational awareness programmes.

**Greater understanding** of falconry as a part of Arab cultural heritage.

### To Hold:

**Regional and International workshops and conferences** on veterinary aspects, falcon biology topics, falconry and conservation issues.

### To publish:

**Papers** on aspects of falcon conservation, falcons and falconry.

A **biannual newsletter/journal** containing contributions on medical, biological and conservation topics of common interest, new developments and recent medical advances.

### Membership:

Membership is open to any veterinary surgeon, biologist, conservationist or falconer working in the Middle East or any other person interested and contributing in the fields of medical, biological and conservation aspects of falcons and falconry worldwide.

Contributions can be sent to the Editors of FALCO:  
**Dr Nigel Barton and Dr Tom Bailey.**

### Editorial address:

Dr Nigel Barton  
P.O. Box 19, Carmarthen  
SA33 5YL, Wales, UK  
Tel: (0044) 1267 253742  
Fax: (0044) 1267 233864  
E-mail: [nigel-barton@easynet.co.uk](mailto:nigel-barton@easynet.co.uk)  
[drtombailey@hotmail.com](mailto:drtombailey@hotmail.com)

### FALCO online

Previous issues of FALCO can be referred to at:

[www.falcons.co.uk/MEFRG/](http://www.falcons.co.uk/MEFRG/)

FALCO relies on articles being submitted by people working in many different areas. We have had great support over the years and would like to encourage continued submission of papers, abstracts, letters and photographs for publication. The newsletter now has a wide readership in many different countries and because of its practical and up-to-date subject matter, it is a useful source of information. It targets those people directly involved in falcon research and management and more importantly it reaches those people who make the decisions. Writing about conservation issues is all very interesting, but unless it influences country representatives at the highest levels, then it remains an interest rather than a priority in the world's current economic and political climate.



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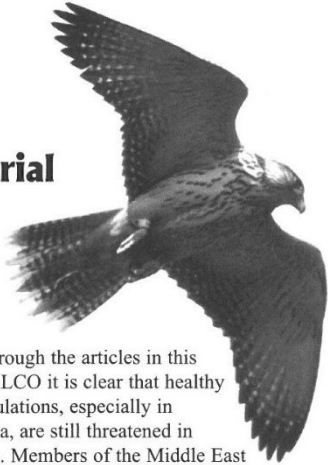


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## Editorial



Looking through the articles in this issue of FALCO it is clear that healthy falcon populations, especially in Central Asia, are still threatened in many ways. Members of the Middle East Falcon Research Group working in the field constantly provide current information on situations as they happen and without these sources of information most of us, including policymakers and organisations would remain oblivious to these serious threats to wildlife. At this very moment, raptors including Sakers, are being poisoned by treated grain in Mongolia, not directly, but because voles are in competition with herders' livestock for vegetation. It seems obvious to us what the consequence of poisoning voles will be as predators higher up the food chain feed from them and consequently die. However, we cannot expect a nomad on the steppe to have the basic biological knowledge to understand these problems. We could, however, expect the manufacturers to take greater responsibility for what they sell. The irony of it all is that by poisoning the voles, they are removing the natural mechanism which to a certain extent controls the vole populations at no cost. But this would not provide an opportunity for business.

There are two issues which should and could be addressed here. In many countries of the world there is a need for basic biological education and awareness. Where better to start than in the schools. Educate the children, some of whom will in future live off the land, others might work in departments where environmental policies are an issue. Most will at some stage in their lives produce children of their own to whom they can pass down the importance of caring for the environment in which they live and on which they depend. In terms of funding, good education is one of the most cost-effective ways of achieving practical improvements for conservation.

The second issue is much more difficult. Selling grain is a business and businesses have the intention to make money. Most business, where public distribution is required, are supported by governments. Unless the governments themselves take a responsibility for their environment then incidences such as the spreading of poisoned grain will continue to happen. Whether we are talking about Central Asia or Europe there is a common problem. There is not enough transfer of information between those in decision-making positions and those on the ground who know what is actually happening. How many millions of dollars have been spent and are still spent by large development organiza-

tions on writing reports on situations and proposals for projects, when most of the time the answers are already known by researchers working in the country. Think how much further ahead we would be if that money had been directed straight at the problem, the people and the environment in need. Practical biology, ecology and environmental studies are certainly a case where the benefits of governmental departments, research organizations and universities collaborating and working together, add up to considerably more than the sum of the individual parts. It is important that we don't cover old ground but direct funding and resources to present-day issues and areas most in need.

We are currently collaborating with CITES regarding the importation of captive-bred birds to the UAE. Representatives of the Middle East Falcon Research Group and the Environmental Research and Wildlife Development Agency (ERWDA) recently held talks with CITES Secretariat. The outcome is of obvious importance but equally important is the knowledge that two organizations, one largely administrative, the other heavily involved in field biology and conservation issues, are working together to resolve a situation which neither could satisfactorily solve on its own. Organisations wield the power, but it is the researchers who have the facts and both need to work hand in hand.

We'd like as always to thank those who have written articles for this 20th issue of FALCO. We regularly receive positive comments about the newsletter, most of them complementary, and we are grateful to those who continue to support the Middle East Falcon Research Group. The newsletter is distributed to many different countries and is proof of the large amount of work which is being carried out on aspects of falcon and raptor biology. It is read by people in policy-making positions, it does influence awareness and we hope that it ultimately benefit raptors and the environment.

The Editors



## Saker Falcon In Pribaikalsky National Park (PNP)

V. Ryabtsev,  
Chief of Scientific Department of PNP  
P.O. Box 185, Irkutsk 665920 Russia  
E-mail: Pribpark@angara.ru

PNP is situated on the western coast of Baikal (Irkutsk Province, Russia). It occupies 4180 sq m. of mountain territory, nearly 1100 sq m of which are relict "island" steppes (surrounded by taiga as zonal type of vegetation). No other Russian national park or nature reserve has such a large area of steppe. This is the northern border of large steppe massifs around Lake Baikal and here, at 53° north and 107° east is the most northerly nesting population of *Falco cherrug* and the only group in the large expanse of the Baikal depression.

At the beginning of the 1990's there were about 10 nesting pairs of saker here and the number decreased to 1 - 2 pairs by 1999. Between 1980 and 1999 the number of saker nesting pairs in the entire Irkutsk Province has decreased from 100 to 10 - 20 nesting pairs. The reason for this is illegal trapping of these birds (Ryabtsev 2001).

Unfortunately, steppe areas within the PNP are not excluded from agricultural use. The conservation measures for steppes within PNP are less than for forests. Nevertheless, the protection service of PNP was informed about illegal trapping of falcons. In 1999 the inspectors discovered two groups of Syrian trappers in PNP. At the time it was not possible to arrest them. In September 2001 together with inspectors of PNP, I twice apprehended the group of 4 Syrian trappers in the steppe part of PNP. They had established a temporary camp in Aya bay of Baikal.

It is difficult to quantify the number of falcons which have been caught by this group. But it is known that from July to the beginning of December 2001 these Syrians were continually travelling in the steppe region of PNP. They

have purchased large quantities of domestic pigeons in settlements. As a result, in September there were pigeon flocks near each herder camp (previously there were absolutely no pigeons in these areas).

Despite being watched by us and aware of the penalties for falcon trapping, the group would not leave the Baikal steppes. This area of steppe appears to be particularly favorable for saker falcons. There are the largest numbers of *Citellus undullatus* here - the main food for falcons, because the steppes of PNP are still under traditional live-stock grazing. Although the number of nesting falcons in PNP is small, there appear to be many single birds living here during the summer when there is high food availability. However it's occurrence is still much rarer than on the Mongolian steppe for example (Ryabtsev 2001). I believe that Syrians are especially interested in the Baikal steppes because of the high quality of falcons which live there. Gyrfalcons (*Falco rusticolus*) also move to these steppes in winter.

For the effective protection of Saker Falcons in PNP special efforts are required. There must be regular raids by PNP inspectors and police on these steppes. Complex controls and monitoring are required in local settlements. Strong co-operation is required at all levels of government and administration. Only then can we make an impact on the illegal trapping which is going on in the Pribaikalsky National Park.

### References:

Ryabtsev, V.V. (2001) Saker Falcon in the Baikal region In: Potapov E., Banzragch, S., Fox N. and Barton N. (Eds) Saker Falcon in Mongolia: Research and Conservation. Proceedings of the 2nd International Conference of the Middle East Falcon Research Group. pages: 58- 63.

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## Problems of Conservation of Falcons in Uzbekistan

B.B. Abdunazarov and M.A. Atadjanov  
Institute of Zoology  
A. Niyaziva St.1,  
Tashkent, Uzbekistan

Eight falcon species are reported from Uzbekistan. Five of these are breeding species. Three of these, the Saker (*Falco cherrug*), Barbary Falcon (*Falco pelegrinoides*) and Lesser Kestrel (*Falco naumanni*) are in the Red Data Book of the Republic of Uzbekistan.

In Uzbekistan, the number of Saker Falcons is estimated at 100-150 pairs. There is a current decline in numbers in



easily accessible areas. The main factors affecting the status of this species are poaching and trapping of these species for commercial purposes. This process has been observed since the early 1990's. During the same period there has been an increase in the numbers of these species using power pylons as nesting sites. They have a higher level of protection and have spread across the steppes.

Conservation measures currently in operation for the Saker Falcon in Uzbekistan include the protection of their territory within the nature reserves and a captive breeding program for subsequent release into the wild. We aim to establish a captive breeding population of 30 pairs which constitutes about 20% of the number breeding in the wild. Over a period of 7 years, approximately 100 individuals have been produced of which 50 were prepared for release into the wild.

Researchers estimate the number of Barbary Falcons at less than 35 pairs. Most of these are nesting in the south of Uzbekistan. Population numbers are higher in September prior to migration. The captive breeding program is establishing a captive population of these species for future production.

The status of the lesser Kestrel is fairly stable and in some regions there is a population density of 100 pairs per 10 km<sup>2</sup>. Fluctuations in numbers depend on the prey base of rodents.

Other measure to protect falcon species in Uzbekistan include the improvement of the legal system prohibiting trapping, import and export from Uzbekistan.



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## Mass mortality of birds in Mongolia

**D. Batdelger**  
birdbdr@yahoo.com

Dashnamjilyn Batdelger is biologist at the Mongolian Museum for Natural History in Ulaanbaatar and president of the Mongolian Society for Bird Conservation. During a 3-week trip in May 2002 to the central aimaks (provinces) of Mongolia, Batdelger found large numbers of dead or dying Demoiselle Cranes (*Anthropoides virgo*). In the vicinity of Zegst-Nuur alone, a small steppe lake, more than 60 dead cranes were found and examined. The number of dead cranes at larger steppe lakes was much higher. During the trip it was regularly observed that birds of prey were feeding on dead cranes. Therefore, many Black Kites (*Milvus migrans*), Steppe Eagles (*Aquila nipalensis*), and Black Vultures (*Aegypius monachus*) are threatened by acute poisoning. The Black Vulture is already a globally threatened species.

The first poisoned kites were found by Batdelger at the end of his trip. Additionally threatened are species which are feeding on grains such as the Mongolian Gull, of which more than 50 dead individuals were found at Zegst-Nuur alone, as well as Rooks, Daurian Jackdaws (*Corvus dauricus*) and other species. Preliminary analysis of stomach contents showed that the Demoiselle Cranes died due to the consumption of poisoned grains. The background is a large scale experiment, carried out by the Mongolian Ministry for Agriculture. It is intended to reduce voles (*Microtus sp.*) by spreading poisoned grains from aeroplanes. The poisoned bait was consumed by cranes arriving in Mongolia from their wintering grounds in May. Batdelger thinks that the pesticide used might be DDT from old stock from the pre-transition period. Many nomads living in central Mongolia reported further cases of large numbers of dead cranes and it is estimated that



hundreds and probably even thousands of cranes died. Batdelger is carrying out public awareness work on environmental protection in Mongolia. But with limited funds and manpower the task is too difficult to be carried out by his small organisation alone. By spreading news on this environmental catastrophe Batdelger hopes to receive some feedback in the form of interventions or protest from international organisations, which will hopefully lead to some changes in the attitude towards conservation by Mongolian politicians.

**Further report on poisoning by:**

**E. Potapov**  
**The Falcon Research Institute**  
**Carmarthen, UK**

It looks as if the mass mortality of cranes, as well as other birds, most notably rodent-eating birds is true, and on an unprecedented huge scale. With an ornithological team led by Prof. Sumya and Magister Gombobaatar from the Mongolian State University, we also observed mass poisoning of various birds and mammals in April and early May in central aimaks of Mongolia. Golden Eagles

(*Aquila chrysaetos*), Saker Falcons, Upland Buzzards (*Buteo hemilasius*), Daurian Jackdaws, Herring Gulls (*Larus argentatus*), Corsak and Red Foxes. Many granivorous passerines were found dead. We have dissected several individuals - the picture is always the same - vast haemorrhage in the cranium and coronary veins, blood clots in the mouth in mammals and eagles.



Poisoned adult female Saker

Demoiselle cranes were found dead shortly after they started to arrive during spring migration. I guess Mr. Batdelger found one of the poisoning hotspots, however our estimation is that the poison may have only reached a small proportion of migrating cranes. The breeding density in our study areas seem to be stable. However we might have missed something. We will report more at the end of the season.

The local herders have been receiving a huge amount of poisoned grain from the local authorities and have been spreading the grains by hand in areas which have a high density of voles. There are no directions as to the type of

grain, what it contains or indeed how it should be safely spread. The poison is distributed by soum (regional settlement) authorities and they receive them from provincial (aimak) authorities in rations (10 tons per soum).

Huge budget money was spent to import the poison from China (where similar cases have been reported). Local herders who have received poisoned grain have been keeping it in their tents (gers) where they store the poison with their own food. Several human deaths have already been reported (Undeer Songin Newspaper and radio broadcast), and children have been taken to hospital.

Its action does not resemble the zinc-based poison, which was previously sprayed from aeroplanes to control voles. The chemical used in the poisoned grain seems to be a persistent poison which is transferred between organisms along the trophic chain.

The scale of the poisoning is huge and covers at least 2/3 of Mongolia and coincides with the area of large numbers of Brandt's Vole (*Microtus brandti*). I reported

the case to the Ministry of Nature and Environment on 17 May 2002, however I have not yet heard about their course of action. The application of poison is usually recommended in early spring, as it is considered that you cannot influence the vole numbers in summer. Mr. Khoroldavaa, the Head of the Department of Natural Resources at the Ministry of Nature and Environment, listened carefully and promised swift action. Prompt action must be taken now to prevent this catastrophic occurrence causing even more mortality amongst the wildlife of Mongolia.



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## Recent data on Saker trapping pressure

**N.W.H. Barton**  
**Falcon Research Institute,**  
**P.O. Box 19, Carmarthen,**  
**SA33 5YL, U.K.**  
**Tel: +44 1267 253742; Fax: +44 1267 233864**  
**Email: office@falcons.co.uk**

The Saker range is swiftly shrinking, and by now it has been reduced to two populations: western-central European and Siberian-Mongolian population. East Ukraine, Central Kazakhstan and Chinese populations have disappeared or are severely overexploited. The most rapid declines have been in European and Kazakhstan populations. Collapse of Chinese populations has not been documented. Causes of decline include irreversible habitat loss to agriculture, man-made reductions in small mammal

populations (see article on poisoning in this issue) and legal and illegal trapping at nests and during the autumn.

The historical range has reduced and fragmented. The sub-species *cherrug* is now fragmented and is not adequately replacing itself. The *milvipes* sub-species is under quasi-legal harvest in Siberia. In Mongolia, largely due to Buddhism, the nests are left alone and production is good, but trappers are increasingly concentrating on this reservoir. China has already been heavily decimated by human pressure; most of the falcons trapped there are of northern origin. Previous estimates for China of 20,000 pairs have recently been revised to about 300 pairs! The Kazakhstan population has shown how the Saker can collapse. Unless the harvest is reduced, the same will happen to the *milvipes* population in perhaps 5 years.

The major users of wild-caught Saker Falcons in the Middle East are Saudi Arabia, Qatar, Bahrain, Kuwait and the United Arab Emirates. The most reliable source of data on numbers of trapped falcons is from the falcon veterinary hospitals in these regions. All of the data shown here are for Saker falcons only (no other species included). It is assumed that the majority of falcons seen for the first time in the falcon hospitals are falcons trapped that same year. This is certainly the case for juvenile birds which account for 80 % of cases. The hospitals from which we have accurate information are the Fahad bin Sultan Falcon Center (Riyadh), Qatar Falcon Center (Qatar), Dubai Falcon Hospital (Dubai), Abu Dhabi Falcon Hospital of the Environmental Research and Wildlife Development Agency, Abu Dhabi. We have estimated numbers for Bahrain and Kuwait.

Although these hospitals provide us with an estimate of wild-caught Sakers, it is only a minimum estimate. Some falcons within the Middle East are never seen at a veterinary hospital and some of the hospitals have been established recently so there will be a period of time before numbers of falcons stabilise to allow a more accurate estimate of annual turnover. The Fahad Bin Sultan Falcon Center is one veterinary hospital in an enormous country. Within 4 years of the hospital opening the number of new sakers seen each year has risen from 484 to 1727 individuals and will continue to rise. The UAE is currently introducing a registration scheme for all falcons in the Emirates. Over the next few years this should also provide more data on numbers of imported species. Although the UAE itself is the smallest user of wild-caught falcons, having encouraged the use of captive-bred falcons, to reduce pressure on wild populations, it is still the main trading region.

**Table 1.**

DUBAI	1993-1994		1994-1995		1995-1996		1996-1997		1997-1998		1998-1999		1999-2000		2000-2001	
	Ad	Juv	Ad	Juv	Ad	Juv	Ad	Juv	Ad	Juv	Ad	Juv	Ad	Juv	Ad	Juv
Male	26	112	27	73	12	53	15	77	17	72	1	12	3	17	2	25
Female	225	929	242	623	222	473	208	563	217	426	70	122	40	90	13	103
Sub-Total	251	1041	269	696	234	526	223	640	234	498	71	134	43	107	15	128
<b>Total</b>	<b>1292</b>		<b>965</b>		<b>760</b>		<b>863</b>		<b>732</b>		<b>205</b>		<b>150</b>		<b>143</b>	

Table 1 shows numbers of saker falcons seen in Dubai Falcon Hospital each year. All data are for different individuals. None of the birds counted in each year are repeats from the same year or from previous years.

Up to and including 1997-1998, the hospital was open to the general public with many falconers also visiting Dubai from Abu Dhabi. Abu Dhabi did not have its own public hospital until 1999. In 1999 the Environmental Research and Wildlife Development Agency opened its public hospital and the same year the Dubai hospital reduced the

numbers of public clients. This probably explains the sudden drop in Saker numbers in Dubai between 1997-1998 and 1998-1999. For this report, data from Dubai and Abu Dhabi are combined.

**Table 2.**

ABU DHABI	1999	2000	2001	2002 incomplete	Total 1165
Age and sex combined	363	418	311	73	

Table 2. Numbers of saker falcons seen in Abu Dhabi Falcon Hospital each year. Data on age and sex ratios is unavailable.

When the hospital data for Dubai and Abu Dhabi are combined for the past few years, they see between 450 and 600 new Sakers each year. Between 1993 and 1995 they were seeing more than 1000 sakers per year. The probable reason for this reduction is that many more people in the UAE nowadays purchase captive-bred falcons. The biggest single user of Saker falcons is Saudi Arabia. Traditionally the Saker has been the falconry bird of choice. Whereas captive-bred falcons are making a definite impact within the UAE, there is currently no such change within Saudi Arabia.

**Table 3.**

RIYADH	1998-1999	1999-2000	2000-2001	2001-2002
Female	482	1027	1610	1652
Male	-	3	10	7
Unknown	2	3	27	68
<b>Total</b>	<b>484</b>	<b>1033</b>	<b>1647</b>	<b>1727</b>

Table 3. Numbers of saker falcons seen in Prince Fahad bin Sultan Falcon Center (Riyadh) each year. At present, data on age ratios is unavailable.

The Riyadh data raise several important points when it comes to estimating Saker numbers:

1. There are many falcons within regions of Saudi Arabia

which are never seen in a hospital.

2. The numbers seen in Riyadh will undoubtedly increase over the next few years.

3. Relatively few of the falcons seen in Riyadh are duplicate counts of those seen in the Dubai and Abu Dhabi hospitals.

4. Annual mortality and losses are high which means that a high proportion of falcons will continue to be replaced each year.

5. Reliable sources suggest that the minimum number of Sakers entering the Kingdom of Saudi Arabia annually could be as many as 4000 individuals.

#### Sex ratios of wild-caught Sakers

The traditional quarry species for saker falcons in falconry is the houbara bustard (*Chlamydotis undulata*). Female Sakers are approximately one third larger than male

Sakers. The size of the houbara dictates that only female sakers are suitable to catch them. The effect of this can clearly be seen from the data. In Riyadh over the past 4 years, 97.5 % of the Sakers seen were females. Given that Saudi Arabia is the biggest user this has serious implications for wild populations.

#### Ratio of adult to juveniles

At the moment it is difficult to give a reasonable estimate for this because we only have detailed data for Dubai. Table 4. The number of adult males and adult females as a percentage of the total number of Sakers seen each year.

Table 4.

	93-94	94-95	95-96	96-97	97-98	98-99	99-00	00-01	MEAN
<b>ADULT</b>									
Male	2.0	2.8	1.6	1.7	2.3	0.5	2.0	1.4	1.8 %
Female	17.4	25.1	29.2	24.1	29.6	34.1	26.6	9.1	24.4 %
<b>JUVENILE</b>									
Male	8.7	7.6	7.0	8.9	9.8	5.8	11.3	17.5	9.6 %
Female	71.9	64.6	62.2	74.1	68.0	65.4	71.3	72.0	68.7 %

We have no data from Kuwait. We know there are Kuwaiti trappers active in many countries. We also know that there are many falcons being kept in Kuwait for falconry and that there are falcon markets in Kuwait. Very few of the falcons kept in Kuwait are taken to hospitals in other regions. The general opinion amongst the established falcon hospitals is that a reasonable estimate for Kuwait is at least 500 new saker falcons each year. Qatar Falcon Center has been open only one year and saw 539 Sakers during this time. We have put a conservative estimate of 1000 sakers for Qatar. On the basis of the above information, estimates for the total number of wild-caught Sakers entering different countries of the Middle East each year are given in the table below. These figures give an indication of trapping pressure.

#### Estimated numbers of Saker Falcons trapped annually in the Middle East:

Table 5. Estimates for annual Saker demand.

COUNTRY	SAKER NUMBERS
Saudi Arabia	4000
Qatar	1000
Bahrain	500 - 1000
Kuwait	500 - 1000
UAE	500 - 1000
<b>TOTAL</b>	<b>6500 - 8000</b>
<b>5% mortality factor added</b>	<b>6825 - 8400</b>

As with any bird species being trapped and exported there will always be mortalities. Using a 5% mortality factor, there could be 6500 - 8500 sakers trapped from the wild each year. In order to establish the sustainability of this level of trapping, we need to estimate the proportion of males, females, adults and juveniles. We also need to know the breeding population size for each country and the numbers of falcons being taken from each of these countries.

#### Estimated ratios for age and sex

(all values based on the minimum trapping level of 6500 with no mortality factor included)

Using the data from Dubai, we estimate that 20% of all trapped falcons are adults and 80% are juvenile falcons.

#### Saudi Arabia (4000)

For Riyadh 98.1 % of the Sakers seen each year were females.

An estimated 780 are adult females and 3140 are juvenile females. (N.B. The figure might be slightly higher allowing for the number of birds where sex is unknown).

#### Qatar (1000)

We estimate that 90 % of the falcons seen here are female and that there is a ratio of 20% adult to 80% juvenile. Therefore we estimate 900 females

and 100 males. We estimate 720 juvenile females and 180 adult females; 80 juvenile males and 20 adult males.

#### Bahrain (500 minimum)

We estimate that 90 % of the falcons seen here are female and that there is a ratio of 20% adult to 80% juvenile.

Therefore we estimate 450 females and 50 males:

1. An estimated 360 juvenile females and 90 adult females.
2. An estimated 40 juvenile males and 10 adult males.

#### Kuwait (500 minimum)

We estimate that 90 % of the falcons seen here are female and that there is a ratio of 20% adult to 80% juvenile.

Therefore we estimate 450 females and 50 males:

1. An estimated 360 juvenile females and 90 adult females.
2. An estimated 40 juvenile males and 10 adult males.

#### United Arab Emirates (500)

In the UAE the ratios are as shown above in this report.

These give estimates for the UAE of:

1. An estimated 360 juvenile females and 90 adult females.
2. An estimated 40 juvenile males and 10 adult males.

Table 6. Summary table for the above data on age and sex. The table shows minimum estimates with countries combined.

	ADULTS	JUVENILES
<b>FEMALES</b>	<b>1230</b>	<b>4940</b>
<b>MALES</b>	<b>50</b>	<b>200</b>
<b>TOTAL</b>	<b>6420</b>	

#### A MINIMUM 6400 SAKERS ANNUALLY ARE TRAPPED AND EXPORTED TO THE MIDDLE EAST.

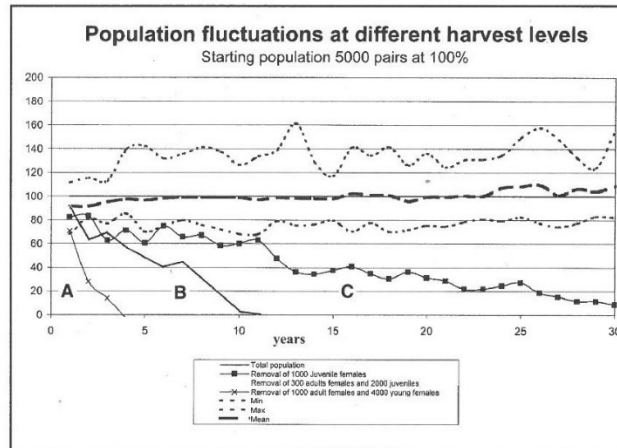
Potapov (unpublished 2002) has calculated a few modelling scenarios using an estimated world population of 5000 breeding pairs of Sakers. The following scenarios were analysed using the model and the effects of the various harvesting scenarios are shown in Figure 1:



A) 1000 adult females and 4000 juveniles removed from world population annually

B) 300 adult females and 2000 juveniles removed from wild population annually

C) 1000 juvenile females and no adults removed from wild population annually



## Poisoning by acetylcholinesterase inhibiting pesticides in free-ranging raptors: a case reported from Saudi Arabia

S. Ostrowski and M. Shobrak  
National Commission for Wildlife Conservation and Development, National Wildlife Research Center, PO Box 1086, Taif, Saudi Arabia.

Corresponding author: [ostrowski@nwrc-sa.org](mailto:ostrowski@nwrc-sa.org)

Poisoning of raptors by acetylcholinesterase (ChE) inhibitors, particularly organophosphorous and carbamate compounds are frequently encountered in Europe (Keymer *et al.* 1981; Lumeij *et al.* 1993). Although secondary poisoning with ChE inhibitors of scavenging birds of prey feeding on poisoned birds and rodents has been documented in Israel (Mendelsohn & Paz 1977), reports of pesticide poisoning of free-ranging birds in the Middle East remain rare, being most probably overlooked. Recently we have reported a case of pesticide poisoning in a free-ranging lappet-faced vulture (*Torgos tracheliotus*) which to our knowledge was the first documented case of pesticide poisoning of a bird of prey in Saudi Arabia (Ostrowski & Shobrak 2001). This article summarises a number of points that may help veterinarians in the Middle East recognize and treat this intoxication in birds of prey.

Diagnosis of ChE inhibiting pesticide poisoning in the live bird is usually based on history, clinical signs and depressed plasma cholinesterase levels (Meerdink 1989).

### History

In the Middle East a history of pest control pesticide spraying over crop fields or over entire portions of territories for locust control must draw the attention in case of abnormal mortality with ataxic symptoms in free-ranging raptors. In the case we described in a free-ranging lappet-

faced vulture two organophosphorus pesticides, fenitrothion and chlorpyrifos, had been used to control outbreak of locusts. The recommended rate of fenitrothion application (250 to 300 g/ha) was near that shown to cause mortality in birds (Steedman 1988) and chlorpyrifos, at a rate of 250 g/ha, might also pose a hazard to migrating birds (Smith 1987).

### Clinical signs

ChE inhibiting pesticide poisoning in raptors appears clinically different than is typically described for mammals (Porter 1987). Clinical signs are not pathognomonic. They include ataxia, spastic nictitans, a detached attitude, inability to fly and occasionally convulsions (Dumoncaux & Harrison 1994). Birds are 10 to 20 times more susceptible to ChE inhibitors than mammals, and young animals appear to be even more susceptible (Humphreys 1988).

### Laboratory diagnosis

Organophosphate and carbamate compounds owe their toxicity to their ability to inhibit ChE, the enzyme that hydrolyses acetylcholine. Theoretically organophosphate and carbamate poisoning can be diagnosed by measuring plasma cholinesterase activity. In practice however definitive diagnosis can be difficult to perform because very little has been published on normal plasma cholinesterase values in raptors (Porter 1987; Hill 1988), analytical method used could markedly affect the results (Fairbrother & Bennett 1988) and other factors (end-stage liver disease, heavy metal poisoning) can also depress plasma ChE activity (Hill & Fleming 1982).

It is therefore important to duplicate analyses in the same laboratory and test concomitantly a negative control bird

(long-term captive) preferably of the same species. In the case we described ChE activity was depressed by 245% compared to the value 20 days after treatment, and by 290% compared to the value measured in a captive griffon vulture (*Gyps fulvus*) tested the same day.

#### Treatment

Specific treatment relies on the administration of atropine sulfate that blocks the muscarinic effects at the nerve synapsis. Dosage of 1% atropine sulfate administered intramuscularly is 0.5 mg/kg (Porter 1993). A higher dosage can be used according to the severity of clinical signs. We used about 1 mg/kg upon arrival as an initial dose in the lappet-faced vulture we treated, and then 0.5 mg/kg at day 3. Improvement was immediate and spectacular. Other symptomatic and supportive treatment should also be provided.

#### Conclusion

Populations of India's commonest *Gyps* vultures have recently dramatically declined due to a mysterious disease (Prakash 1999). Sick birds appeared lethargic with drooping heads and wings, all symptoms compatible with a ChE poisoning in bird. However, recent investigations seem to have ruled out anti-ChE pesticides as the cause of the disease and instead point towards an infectious cause (Prakash et al. 2002). People have conceived that the disease that seem to affect all *Gyps* vultures could spread from South Asia throughout the Middle East and the Old World (Prakash et al. 2002). It is important therefore that veterinarians throughout the Middle East play a role of epidemiological sentinels and investigate to the best of their capacities any sick vulture with a lethargy syndrome. Should any case arise, anti-ChE poisoning will have to be ruled out.

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# Catastrophic Declines of Griffon Vultures in India

A. A. Cunningham<sup>1</sup>, D. Pain<sup>2</sup> & V. Prakash<sup>3</sup>

<sup>1</sup>Head of Wildlife Epidemiology, ZSL Institute of Zoology, Regent's Park, London, NW1 4RY, U.K.

<sup>2</sup>Debbie Pain, Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, U.K.

<sup>3</sup>Principal Scientist, Bombay Natural History Society, Hornbill House, Shaheed Bhaagat Singh Road, Mumbai 400 023, India

In 1996, Dr Vibhu Prakash of the Bombay Natural History Society (BNHS) published a paper (Prakash 1999) describing the rapid and marked decline of the Indian white-backed (*Gyps bengalensis*) and Indian long-billed (*Gyps indicus*) vultures in the Keoladeo National Park (KNP, World Heritage Site), Rajasthan, India. Nation-wide surveys conducted by the BNHS, with support from the Royal Society for the Protection of Birds (RSPB), showed that similar (>90%) declines of both species had occurred throughout India between the early 1990s and 2000 (Prakash *et al.* in press). Both affected species were once regarded as very common in India, but now they are listed as critically endangered by the IUCN.

Dr Prakash had noted that many *Gyps* spp. vultures in and around KNP appeared sick with intermittent, often prolonged periods of head drooping. Such birds inevitably died and the mortality rate of these species in KNP was extremely high. During the nation-wide surveys, vultures across the country appeared lethargic and sick with drooping heads. Dead birds were frequently found.

Other species of scavenger, including non-*Gyps* vultures were not affected at KNP. This situation held true for all areas surveyed throughout India. In fact, apart from the rapidity and extent of the declines, the most remarkable aspect is that only *Gyps* spp. appear to be affected.

Initially poisoning, persecution and a lack of food were considered as possible causes of the declines, as is often the case for vulture mortality incidents elsewhere. As reported for KNP (Prakash 1999), food appeared to be abundant throughout the country and few livestock carcasses had attendant vultures (Prakash *et al.* in press). Examination of vulture tissues for exposure to pesticides and other poisons have, so far, drawn a blank (Oaks *et al.* 2001; Rahmani & Prakash 2000) and there is no evidence or culture of persecution of vultures in India. The genus-specific nature of the causative agent, along with the lack of a biogeographic patchiness (e.g. similar declines in urban vs rural, protected areas vs agricultural areas) to the vulture declines and mortality, implies that the cause is likely to be more insidious than any initially proposed. The pattern is consistent, for example, with that of an infectious disease epidemic, such as occurred in ungulates when rinderpest was introduced into Africa.

Prompted by these dramatic and worrying findings, in

2001 the RSPB, BNHS and ZSL's Institute of Zoology obtained grant funding from the UK Government's Darwin Initiative for the Survival of Species and initiated a systematic investigation into the cause of the vulture declines. This has involved setting up a Vulture Disease Investigation Laboratory as a sub-section of an established avian diagnostic laboratory in India, and obtaining freshly-dead vultures for post mortem examination. Obtaining such carcasses has not been an easy affair, and a large number of people have spent a great deal of time occupied with this aspect of the work. To date, we have examined 30 carcasses in detail, comprising both long-billed and white-backed vultures, adults and juveniles. We have been very fortunate in this endeavour to have access to the staff and facilities of the Poultry Diagnostic and Research Centre (PDRC) in India, without which the provision of follow-up diagnostic tests would not have been possible.

The results so far have shown that many of the birds died with evidence of enteritis and of severe renal and visceral gout. Although renal gout is often attributed to kidney disease, in these cases the gout has been extensive and acute (or peracute) - i.e. occurring only a few hours (or less) before death. This condition is, therefore, a consequence of the primary disease and not the disease itself. It is most likely a response to terminal dehydration of the affected bird, although we have not yet been able to prove this. Apart from the gout and enteritis, there has been remarkably little to see at gross post mortem examination of affected vultures, although microscopic tissue examination revealed that lymphocytes (a type of white blood cell) had migrated from blood vessels, often an indication of a reaction to an infectious disease.

In addition to the post mortem investigations, money from the Darwin Initiative is being used to build and staff a "Vulture Care Centre" in India. Sick birds will be housed in this Centre in order to learn more about the disease and to try to bring about a recovery via veterinary interventions. The National Bird of Prey Centre in the UK, specialists in raptor care in captivity, are advisors to this part of the project. Also, for the three years of the Darwin funding, India-wide population surveys and health surveillance will be carried out, so the progress of the problem in India can be carefully monitored. The ultimate aim of this work is to produce a vulture recovery plan.

Although the initial findings from our investigations are tantalising, they do not yet provide an answer as to the cause of the disease killing the vultures. Work is on-going both (1) specifically aimed at identifying a viral cause of the disease, as suggested by the diagnostic investigation so far, and (2) broadly aimed at identifying any possible cause of the birds' demise - infectious or non-infectious. We are keeping our diagnostic minds as open as possible because, although our findings are suggestive of a viral involvement, this may not be the case or, if so, it may only be part of the story.

Another method of investigation we are pursuing, that could be very helpful with our studies, is the comparison of blood samples from the affected wild vultures in India with those of vultures not affected by the declines. Samples from wild-caught Indian vultures now in captivity would be particularly useful in this respect. We are particularly interested in receiving serum samples from clinically healthy old-world vultures (captive or wild). These samples will be stored frozen at the Institute of Zoology, London and kept for use by diagnostic and research laboratories investigating the vulture disease in India, should such samples prove useful. Comparisons between serum samples collected within and outside India may help us to identify the causative agent. For example, if putative infectious agents are found during the course of our investigations, the samples would be tested for evidence of antibodies to that agent and the results compared with serum samples collected from the affected vultures in India. Further details of how to participate with the serum collection can be found on the relevant page of the vulture project web site ([www.vulturedeclines.org](http://www.vulturedeclines.org)).

The dramatic vulture declines observed across India present a whole range of threats, both ecologically and to human health. The absence of such important scavengers will almost certainly influence the numbers and distribution of other scavenging species. For example, as vultures have declined feral dog populations have been reported to have increased massively, with over 1,000 observed recently at a carcase dump in Rajasthan - this could pose many associated disease risks to humans and wildlife, such as rabies.

The international implications of this problem are also very concerning. If it is an infectious disease that is affecting the two resident *Gyps* spp. of vulture in India, it is conceivable that it will spread beyond the Indian borders. The ranges of species of the *Gyps* genus overlap from India through central Asia and the Middle East to South Africa and Western Europe. Griffon vultures are known to travel widely and it is possible that a disease of *Gyps* spp. vultures could spread from South Asia throughout the old world. Already, there are reports of dead vultures and population declines in Pakistan and Nepal. While these reports are also of Indian white-backed and long-billed vultures, other *Gyps* species may also be at risk. Particularly worrying in this respect are recent reports from Dr Prakash of finding small numbers of sick and dead Eurasian griffons *Gyps fulvus* and Himalayan griffons *Gyps himalayensis* on their wintering grounds in India.



Himalayan Griffon

The Birdlife network has been used to alert bird conservation organisations in all of the *Gyps* spp. range states of the problem. We are encouraging organisations throughout range states to closely monitor vultures in their areas. In order to allow comparable results from different countries, standard methods for monitoring and surveillance have been developed and published on the vulture project web site ([www.vulturedeclines.org](http://www.vulturedeclines.org)). Further information about the project may also be found on this web site.

If you would like to support this work financially or in any other way, or if you would like advice or help to start work on monitoring vultures in your area, please contact us.

For pathological investigations and collection of serum samples: Dr Andrew Cunningham - [andrew.cunningham@ioz.ac.uk](mailto:andrew.cunningham@ioz.ac.uk)

For monitoring within India : Dr Vibhu Prakash - [jatayu\\_prakash@yahoo.co.in](mailto:jatayu_prakash@yahoo.co.in)

For monitoring outside India: Dr Debbie Pain - [debbie.pain@rspb.org.uk](mailto:debbie.pain@rspb.org.uk)

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# Asian Vulture Crisis Project: Preliminary results for 2nd breeding season, Pakistan and Nepal, 2001-2002 (June 2002)

The Peregrine Fund  
566 West Flying Hawk Lane  
Boise, Idaho  
USA

Conservation priorities of species in jeopardy often demand a basic understanding of their natural history before effective recovery plans can be implemented. The Peregrine Fund, along with in-country partners the Ornithological Society of Pakistan, Bird Conservation Nepal and others, have been working to understand the population dynamics of Asian *Gyps* vultures to determine the cause and extent of vulture mortality in south Asia since October 2000. Systematic monitoring and diagnostic programs have been in place for two breeding seasons, quantifying vulture productivity and mortality in colonies spaced widely across the subcontinent. This work is being supported by a grant from the Gordon and Betty Moore Foundation, along with other donors.

During the second field season in Pakistan (2001/02), 1218 Oriental White-backed Vulture nests were located at three sites (Dholewala [DW], Toawala [TW] and Changa Manga [CM]) across the Indus Plain. Numbers of breeding pairs have decreased by ~75% at CM, ~37% at DW and ~11% at TW since 2000/01. The study found 487 dead vultures during the 2001/02 breeding season and annual adult mortality rates of breeding birds were ~27% at CM, ~14% (DW) and ~11% (TW). These were similar to mortality rates of the previous season. Prevalence of visceral gout amongst adults and subadults remained high at ~80%. Fieldwork is ongoing and dead vultures continue to be found.

At Koshi Tappu, Nepal, a significant decrease in numbers of breeding Oriental White-backed Vultures was observed (67 nests in 2001 to 12 nests in 2002). Only two out of nine active nests successfully produced fledglings, while sightings of the sympatric Slender-billed Vultures in the area were rare (only two birds seen). Five active Himalayan Vulture nests were located in the Annapurna Range. Numbers of this species appear to have remained stable over the last two decades when compared with previous surveys.

A new *Mycoplasma* species was identified from vulture tissue collected in Pakistan. Nine captive vultures were experimentally infected with tissues from gout-affected vultures and have not so far exhibited clinical signs consistent with sick vultures observed in the field. Interviews with 168 farmers showed that spraying of organophos-



phate, pyrethroid and nitrile compounds on cotton and wheat was intensive and recurrent. The use of organophosphate pesticides in Pakistan has increased significantly over the last decade. Pesticide storage and disposal practices are poor, resulting in run-offs into water systems and a high level of exposure to both humans and animals. Diagnostic studies have not so far established a link between gout in vultures and agricultural pesticides used in the region.

Findings suggest that mortality rates differ between the three vulture colonies, and this has been supported by a corresponding variation in population decline. The decline of vultures in India has coincided with an almost three-fold increase in the use of pesticides in the region over the last decade. Previously common resident raptors such as White-eyed Buzzards and Black-shouldered Kites are now rarely encountered in the Punjab Province, suggesting that *Gyps* vultures may not be the only genus in decline. Temporal and spatial clusters of dead vultures have been located indicating a point source of exposure. This finding, combined with high pesticide consumption in the region supports an intoxication theory, but does not rule out the possibility of a disease agent.

Despite the current political situation in south Asia, The Peregrine Fund and their in-country partners are committed to understanding the cause of vulture mortalities in the region. For more information about The Peregrine Fund's Asia Vulture Crisis Project, please visit [www.peregrine-fund.org/conserv\\_vulture\\_results.html](http://www.peregrine-fund.org/conserv_vulture_results.html)

# The history of falconry in China

Y. Xiaodi<sup>1</sup>, R. Chang<sup>2</sup> and N. Fox<sup>3</sup>

<sup>1</sup> Institute of Zoology, The Chinese academy of Sciences, Beijing 00080, Email: yexd@panda.ioz.ac.cn

<sup>2</sup> 18 Zhangjiaochang, Daqitiao, Xijiekou, Beijing

<sup>3</sup> National Avian Research Center, Wales, UK

The art of falconry is practiced in many countries throughout the world, in Asia, the Middle East, Russia, western Europe, North America and Australia. It is becoming increasingly common as a field sport.

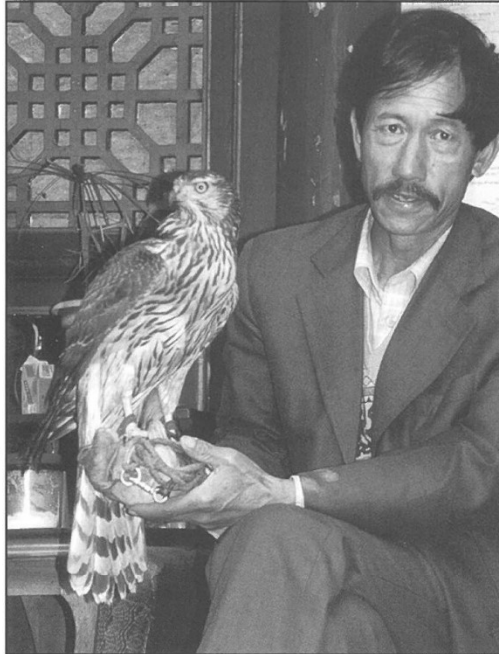
China is the cradle of traditional falconry culture and techniques and the history of Chinese hawking can be traced in the literature back to the central and lower reaches of the Yellow River (Huanghe) valley around 1500 B.C. In Chinese history, the hawking sport has been thriving in the Spring and Autumn Period (740–330 B.C.), Qin Dynasty (221–206 B.C.), Sui Dynasty (581–618 A.D.), Tang Dynasty (618–907 A.D.), Song Dynasty (960–1279 A.D.), Liao and Jin Dynasties (907–1234 A.D.), Yuan Dynasty (1206–1368 A.D.) and Qing Dynasty (1616–1911 A.D.). The hawking technique and culture spread far and wide as far as northern national minorities such as Hui, Serbi, Mongolia, Wusun, in the last stage of Qin, Han and Tang Dynasties, and then spread to Central Asian countries afterwards. In the fourth century, the hawking technique was introduced to western Europe. It spread from China to India during the fifth century and to Japan in 247 A.D.

According to our statistical survey at the end of 2000, the old conventional traditions of hawking are still retained amongst 18 tribes of China, eg the Han, the Hui, the Naxi, and the Uigur. It is estimated that there are 500 falconers in the entire country, who are distributed mainly over northeast, north, northwestern China and Yunnan Province. There are 14 species of hunting birds, primarily Northern Goshawk (*Accipiter gentilis*), Eurasian Sparrow Hawk (*A. nisus*), Japanese Sparrow Hawk (*A. gularis*), Golden Eagle (*Aquila chrysaetos*), Saker Falcon (*Falco cherrug*) and Peregrine Falcon (*Falco peregrinus*). Prey species include Brown Hare (*Lepus capensis*), Woolly Hare (*L. oiostolus*), Red Fox (*Vulpes vulpes*), Tibetan Fox (*V. ferrilata*),

Tibetan Gazelle (*Procapra picticaudata*) and Common Pheasant (*Chrysolophus pictus*).

However, one of the species used in falconry is faced with ecological disruption of its environment as well as persecution. The Saker Falcon is trapped, killed and smuggled; raptors are captured to sell or to eat. They are also poisoned by agricultural chemicals.

Ever since the implementation of the Wildlife Conservation Law of China in 1989, the development of this traditional Chinese hawking culture has encountered many difficulties: raptors are forbidden to be used for hawking and since the deployment of the wildlife conservation movement, hawking is regarded as an illegal activity; the Chinese hawking traditions are gradually being lost; the legal hawking rules and the management procedures have not been published yet; there has never been a thorough review of the Chinese hawking traditions and culture.



In view of this, we think that the following aspects should be taken into account in order to retain the traditional Chinese falconry and hawking: (1) establish a falconer licensing system and conduct management according to law; (2) establish captive breeding of falconry species in China (3) establish a falconers association or falconers club (4) balance wildlife protection with traditional Chinese falconry (5) Set up a falconry museum and training scheme for new falconers (6) Increase research into Chinese falconry

techniques (7) international co-operation to crack down on smuggling raptor species (9) advance ecological research techniques (10) promote the research and collaboration between relevant international organizations and Chinese falconers.

## Acknowledgement:

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# First documented clutch and brood of six in Saker Falcon (*Falco cherrug*).

E. Potapov<sup>1</sup>, D. Sumya<sup>2</sup>, S. Gombobaatar<sup>2</sup>, O. Shagdarsuren<sup>2</sup>, S. Tuya<sup>3</sup>, L. Ochirkhuyag<sup>4</sup> & N. Fox<sup>1</sup>.  
**1.** The Falcon Research Institute, PO Box 19, Carmarthen SA33 5YL, UK  
**2.** Faculty of Biology, Mongolian State University, Ulaanbaatar, Mongolia  
**3.** Center for Environmental Remote Sensing, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba, 263-8522 Japan, E-mail: s.tuya@lycos.com s.tuya@cr.chiba-u.ac.jp  
**4.** The National Remote Sensing Center, The Information and Computer Center, Ulaanbaatar-210646, Mongolia, lochir@yahoo.com

## Introduction and methods

The Mongolian programme of the Falcon Research Institute has been running since 1998. The field team has been led during the 5 field seasons by Prof. D. Sumya and Magister S. Gombobaatar under the supervision of Acad. O. Shagdarsuren. The aim of the scientific programme is to study Saker Falcon biology in Mongolia and to provide the Mongolian government with population data, numbers and breeding rates of the Saker populations to be used in establishing harvesting quotas for falcons in compliance with CITES regulations.

Within study areas, Saker nests were mapped and their breeding performance monitored. There were some nests outside the study areas which were visited but they were not used in calculations of population density. Clutch size, which is considered to be one of the most important factors determining a species breeding rate (Newton 1979), as well as breeding success was determined in as many nests as possible. In total we have data on 188 clutches/broods during the period 1998-2002.

We also examined the possible influence of winter snow cover on clutch size. The snow coverage data was analysed using GIS methods at the Mongolian National Remote Sensing Center of Mongolia. Snow cover in Mongolia has been monitored since 1999 and the snow cover maps are published on the website of the Ministry of Nature and Environment of Mongolia. A combination of meteorological station measurements and mapped snow distribution was used to validate and quantify the satellite data.

## Results

Clutch size in Mongolian Saker falcons (Figure 1) varies significantly across years (ANOVA:  $F=9.59$ ,  $P=0.0000007$ ). Largest clutch sizes were observed in 1999 and 2002. Both winters prior to these breeding seasons were characterised by low winter snow coverage. The winters of 1999/2000 and 2000/2001 are known as severe cold and snowy winters with so-called "zud" conditions. "Zud" conditions means that the ground was covered by ice

formed before snow cover, thus making grazing by rodents and livestock difficult.

The symmetry of the clutch size distribution was not constant across the years (Figure 1). An almost symmetrical distribution was observed in 2001 and 2002 but was skewed towards smaller clutches in all other years, most notably in 2000. During 2002 we found an unexpectedly high proportion of large clutch sizes.

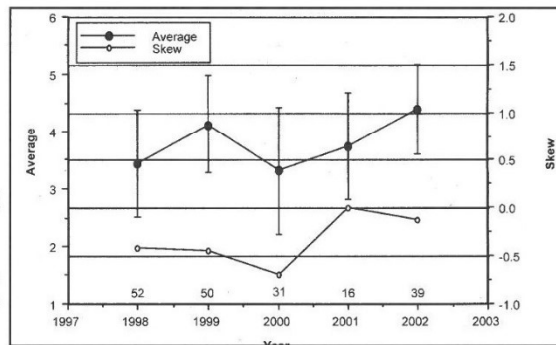


Fig. 1. Clutch size variation across years with S.D. Figures on horizontal axis are sample size.

Snow cover in Mongolia showed significant variation between 2001 and 2002 (ANOVA:  $F=9.36$ ,  $F=0.005$ ) as did clutch size across the years (ANOVA:  $F=6.68$ ,  $P=0.01$ ) (Figure 2).

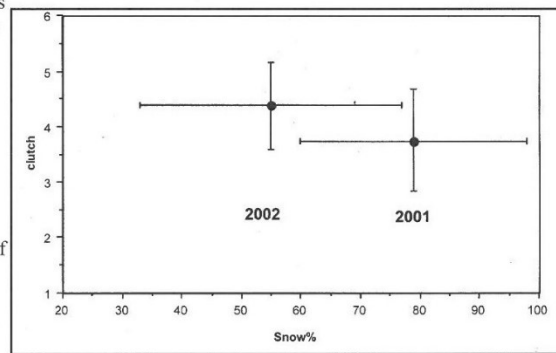
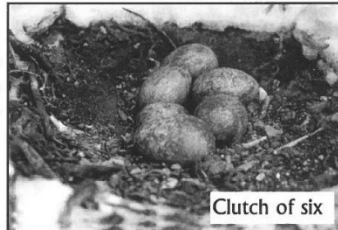


Fig. 2. Clutch size variation plotted against % snow cover.

In 2001 there was greater snow coverage and smaller clutch sizes. In 2002 the snow cover across Mongolia was very unstable and unusually thin. Clutch sizes for 2002 were at a maximum for the whole 5 year period. We have recorded two clutches of 6: six chicks in down in one nest and 6 eggs at a different location 241 km apart.

## Discussion

A clutch of 6 is very big for large falcons. References on Mongolian Sakers (see Shagdarsuren *et al.* 2000 and references) do not mention clutch or brood sizes of 6. Any references to a clutch size of six eggs come from Somov (1897) in his study of the birds of Kharkov district, now in eastern Ukraine, where Sakers are nowadays believed to be extinct. There is no precise information on the real clutch sizes, nor is there evidence in collections to support the occurrence of clutch size of 6. Dementiev *et al.* (1951) mentioned the clutch size of 6 in European Saker *F. cherrug danubialis*, Common Saker *F. cherrug cherrug* and Mongolian Saker *F. ch. milvipes*, however no references were given. Hence we consider that this is the first documented record of a clutch and brood of six.



Vole cycles alone cannot explain variation in clutch sizes. In the studies of 1998-2002 the vole densities varied dramatically across the study areas, however, we failed to find corresponding variation in the breeding rates of sakers, or in the breeding of other birds of prey.

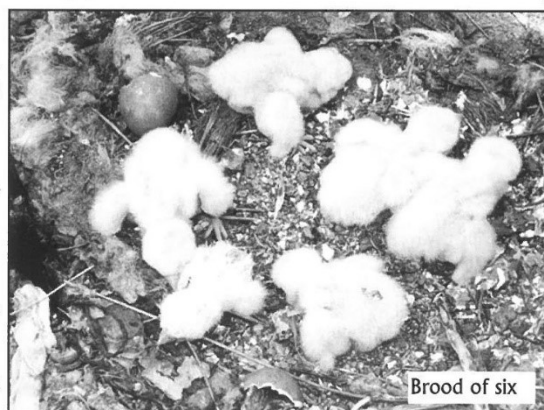
Livestock play an important role in the cycle of Brandt's vole. According to hypotheses of Shagdarsuren, large concentrations of livestock, especially of sheep and goats create overgrazing situations, which are immediately used by Brandt's Vole (*Microtus brandti*) - the main food of wintering falcons in Mongolia as well as those breeding in vole areas. Low vole numbers are associated with low livestock numbers since the Brandt's vole cannot breed in high numbers in tall grass areas (Galushin *et al.* 1999). Sakers might be benefiting from high livestock densities because the domestic animals create overgrazed areas thereby allowing an increase in vole numbers. The voles peak and create super-abundance thus benefiting the falcons. Over-wintering voles usually finish off the pre-rooted parts of the grass thus leaving no grass to the livestock. The voles in high-density areas might severely deplete grass stands in the steppes and thus demonstrate obvious competition to livestock. In the fear that depleted grass might push them to relocate the livestock, local herdsmen are not happy with the voles and often try to reduce the competition by spraying rodenticides. After the livestock has been removed and thus grazing pressure reduced to zero, the grass starts to recover and the cycle repeats itself.

As the Saker in central Mongolia is normally non-migratory (Sumya *et al.* 2001), the amount of snow is crucial for them during the winter. In the years with low snow cover they might achieve good pre-breeding conditions even at moderate vole densities. In the years of deep snow cover they might opt to migrate short distances even at high vole densities. It is well known that high vole densities attract large numbers of Sakers in autumn (Bold and Boldbaatar 2001, Shagdarsuren *et al.* 2001), however nobody reported

large densities of Sakers in these same areas in winter. Thus, snow cover as an obstruction might play a very important role in breeding decisions in Sakers.

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# Simple molecular methods for sexing birds

K.R. Oddie<sup>1</sup> and R. Griffiths<sup>2</sup>

<sup>1</sup>Centre d'Ecologie Fonctionnelle and Evolutive, UPR 9056 CNRS, 1919 Route de Mende, F-34293, Montpellier CEDEX 5, France. Email: gobiology@yahoo.com

<sup>2</sup> DEEB, Graham Kerr Building, Glasgow University, Glasgow G12 8QQ, U.K.

A recent article in *Falco* documented the problem of correctly identifying the sex of birds where it was not possible to do so from physiological appearance (D'Aloia 2002). Molecular sexing techniques were applied to blood samples taken from pale chanting goshawks *Melierax [canorus] canorus* to determine the sex of birds. Although many raptors can be sexed visually by reversed size dimorphism, this is not always conclusive, especially with young birds.

In the case of the goshawks it was desirable to determine the sex of all the birds so that they could be relocated in pairs, and positive identification of sex is of obvious importance for creating potential breeding pairs. It may also be helpful in speeding up trade of birds so they can settle into new homes quicker, and when caring for birds, when one sex may be particularly susceptible to disease or stress. For veterinarians, scientists and ecologists, identifying sex is necessary to monitor sex differences in development. For breeders it can be useful to record the sex of nestlings hatched by females, to create family trees recording the success of pairs and their young (see also Griffiths 2000a).

The last ten years has seen the development of molecular techniques to allow sexing of birds from DNA samples, so that invasive methods such as laparotomy and laparoscopy, or expensive and time-consuming cytological sex identification are becoming obsolete. Sexing from DNA requires only a tiny (2-10ml) amount of blood (e.g. Griffiths *et al.* 1998, Kahn *et al.* 1998) or feathers (Griffiths and Tiwari 1995), which appeals to conservationists, breeders and scientists alike. Furthermore, one quick and simple method has been developed which can be applied universally to all birds excepting ratites. As it is simpler, more efficient, and less expensive than other molecular methods, we explain its functioning here.

Molecular sexing methods rely on isolating and identifying different sized fragments of DNA found in male and female birds. The avian genome (see glossary for scientific terms) has a set number of chromosome pairs called

autosomes; for each pair, one is derived from the mother and one from the father. Two other chromosomes, the sex chromosomes, also exist and are derived in a similar manner. These 'Z' and 'W' chromosomes determine the sex of the bird, as females have a Z and a W chromosome but males have two copies of the Z. This scheme is called 'female heterogamety' because female birds have two different sex chromosomes.

Fragments of DNA from the Z and W chromosome can be identified from a blood sample, so allowing us to determine a bird's sex. A method called PCR is used to identify a specific piece of a gene, the CHD1 gene, found on the sex chromosomes. This gene is highly conserved: homologous have been found in mice (Delmas *et al.* 1993), humans, *Drosophila* and yeast (Woodage *et al.* 1997). It is because of this that the sexing test can be applied to all birds (Griffiths *et al.* 1998), as all bird species contain the CHD1 gene (although there have been problems sexing ratites).



CHD1, like all genes, is made up of 'exons' which are functional pieces of DNA and consequently whose sequence (form) is highly conserved, and 'introns' - DNA with no purpose. In the sexing PCR, a pair of primers (short strands of DNA) identify the CHD1-W gene on the W chromosome and the CHD1-Z gene on the Z chromosome. The chemical properties of DNA allow these primers to make vast numbers of copies of a specific fragment of

DNA within these genes. The primers have been designed especially to locate this exon region and amplify DNA across an intron. The intron size varies between the CHD1-W and CHD1-Z gene and consequently the products of the PCR are two different sized fragments. These fragments can be visualised by standard DNA visualisation techniques that allows discrimination of DNA fragments by size (see box 1). The result is that females have two bands but males only one. An advantage of this test is that it amplifies both CHD1-W and CHD1-Z, which allows positive identification of both sexes. (If a PCR amplified only CHD1-W it would be impossible to designate a null result as a true male or simply a failed PCR reaction).

Currently there are three tests designed to identify sex using this method (see also Griffiths 2000b), two which amplify the same intron (Griffiths *et al.* 1998, Kahn *et al.* 1998) and another which amplifies a different intron still on the CHD1 gene (Fridolfsson and Ellegren 1999). Table 1 gives primer sequences and PCR conditions for reactions. The test used by D'Aloia (2002 and also D'Aloia and Eastham 2000) functions in a similar way, but requires DNA cutting with a restriction enzyme after PCR amplifi-

cation, necessitating the identification of a suitable enzyme and adding extra work, time, expense and opportunity for error. We therefore suggest this more simple test. The choice of primers to use is based initially on study species, and where one test fails another can be performed; for falcon species the 2550F/2718R (table 1) have been found to work well.

### Troubleshooting

When PCR conditions described here are not successful several steps can be taken to remedy the situation. Firstly, the concentration of DNA in the PCR mix may hamper the reaction (usually too much) and it is advisable to try a dilution series of PCRs. If the CHD1-Z product amplifies better than the CHD1-W, try lowering the annealing temperature and/or reducing extension time. Where there is little size difference between the W and Z bands, use a polyacrylamide rather than agarose gel to allow better discrimination between band lengths.

The sexing methods explained here represent an efficient, cheap and reliable means to sex birds and can be carried out in any standard molecular biology lab. For those without access to such facilities, commercial sexing is available, e.g. from University Diagnostics, 90 London Rd, London, SE1 6LN, UK.

### Glossary:

*Autosomes*: non sex chromosomes

*Genome*: entire genetic complement of a prokaryote (organisms with no nuclei) or virus, or the haploid genetic complement of a eukaryote

*Haploid*: a state of having one copy of each chromosome per nucleus

*Heterogametic*: the sex with different morphs of sex chromosomes; in cell division different kinds of gametes are produced in regard to these sex chromosomes (e.g. the male state in humans that produces sperm with X and Y chromosomes)

*Homogametic*: the sex with same morphs of sex chromosomes that produces just one kind of gamete (e.g. females humans that produce ova with X chromosomes)

*PCR (polymerase chain reaction)*: method to amplify rapidly selected DNA segments in cycles of DNA denaturation, primer binding and replication.

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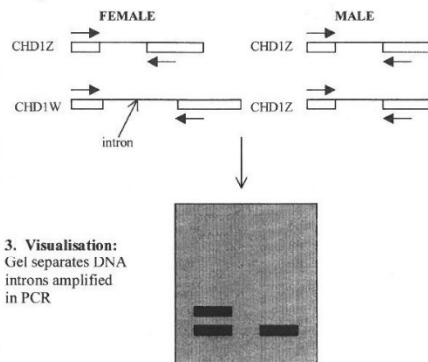
### Box 1: Molecular method of sex determination

#### 1. DNA extraction:

Nuclear DNA is extracted from blood or feathers for use in the reaction.

#### 2. PCR amplification:

Primers hybridise with a region on the highly conserved CHD1 gene and create multiple copies of DNA fragments from the Z and W chromosomes in a polymerase chain reaction (PCR). The area of the CHD1 gene amplified contains introns of different lengths on the Z and W chromosomes.



#### 3. Visualisation:

Gel separates DNA

introns amplified

in PCR

Following amplification, the products of the PCR are run on an agarose or polyacrylamide gel and visualised (e.g. using ethidium bromide or silver staining respectively for different gel types, see Promega, 1996). Females are recognised by the appearance of two bands on the gel, which represent a copy of one intron found on the Z chromosome, and another found on the W chromosome. Homogametic males possess only Z chromosomes and are therefore identified by only a single band on the gel.

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Primers	Sequence	Conditions	Ref
P2/P8	P2 TCTGCATCGTAAATCCTTT P8 CTCCCAAGGATGAG(A/G)AA(C/T)TG	10µl Standard conditions; 94C/90, 30x (48C/45, 72C/45, 94C/30), 48C/60, 72C/300; 3% agarose	Griffiths <i>et al.</i> 1998
1237L/ 1272H	1237L GAGAACTGTGCAAAACAG 1272H TCCAGAAATATCTTCTGCTCC	10µl Standard conditions; 94C/90, 30x (56C/60, 72C/60, 94C/30), 48C/60, 72C/300; 3% agarose	Kahn <i>et al.</i> 1998
2550F/ 2718R	2550F GTTACTGATTCGTCTACGAGA 2718R ATTGAAATGATCCAAGTGCCTG	10µl Standard conditions but no triton, primers at 2pmol, MgCl <sub>2</sub> 1.75mM non passerines or 3mM passerines; 94C/120, 10x (60->50C/30, 72/30-40, 94/30), annealing stage has 1C touchdown from 60C to 51C, 25-35x (50C/30, 72C/30-40, 94C/30), 50C/30, 72C/300; 2-3% agarose	Fridolfsson and Ellegren 1999

Table 1: Primer sequences and reaction conditions for PCR identification of sex for all avian species, except ratites.

## Aspergillosis: Therapy and Prevention in Zoo Animals with Emphasis on Raptors

I. Bailey  
MSc Programme Wild Animal Health  
Zoological Society of London

The Royal Veterinary College, London, UK

### 1. Background

Aspergillosis is a disease of increasing importance in human medicine because of rising numbers of immunosuppressed patients vulnerable to infection (Hill *et al.*, 1995). Aspergillosis was the first mycotic disease to be described in animals, although accounts of an incurable disease of the lungs called 'pantars' by the falconer Latham (1615) pre-date this. Aspergillosis has implications in avian conservation programmes, the first generation of captive breeding projects are often derived from the wild, which are stressed and susceptible to infection. In a review of factors involved in the failure of the translocation and breeding programmes undertaken by the New Zealand Conservation Department of the rare stitchbird (*Notiomystis cincta*), aspergillosis was the single most important cause of mortality (Cork *et al.* 1999).

Aspergillosis is the most common systemic avian mycosis. Of 66 case reports published on aspergillosis from 1970-2000 in exotic species (Veterinary Librarian) 54 (81%) were avian species, 11 (17%) were mammals and 1 was a reptile. Established infections are challenging to resolve and this paper aims to review current approaches to therapy and prevention of aspergillosis with emphasis on raptors.

### 2. Treatment Considerations

#### 2.1. Classification of Aspergillosis

Avian aspergillosis has been classified by Redig (1993a) as 1) an acute form caused by a single exposure to an overwhelming number of spores; 2) a tracheal form; 3) localised granulomas in the air sacs and lungs; and 4) an invasive form which initially affects the respiratory system and then spreads haematogenously and through the air sacs to the rest of the body. The success of treatment depends on location and extent

Table 1. Factors associated with the susceptibility of animals to aspergillosis (Cooper, 2002; Webeser, 1981; Prescott, 2000).

Destruction of normal host microflora by broad-spectrum antibacterial drugs	Environmental factors (e.g. low humidity, dust, decaying organic matter)
Immunosuppression	Species susceptibility (e.g. gryfacons, sea ducks; see Table 2)
Pre-existing disease conditions (e.g. haemochromatosis)	Malnutrition
Treatment with immunosuppressive drugs (e.g. corticosteroids)	Vitamin A deficiency
Exposure to respiratory irritants (e.g. cigarette smoke, ammonia)	Other causes of physiological stress (e.g. recent capture or oiling)

of the lesions and effective treatment can usually only be instituted in the tracheal and semi-invasive or localised forms. Severe cases, where the patient exhibits cachexia, dyspnea and vomiting, are beyond the point of treatment (Redig 1993a). Treatment goals for aspergillosis are 1) removal of lesions that restrict airflow, 2) killing and elimination of the fungus and 3) provision of supportive care (Redig 1981). Unless the lesions are detected early, drug therapy alone is unlikely to be effective because of the thick caseous encapsulation which develops in the airsacs (Forbes 1996). Factors associated with the susceptibility of animals to aspergillosis are presented in Tables 1 & 2.

Table 2. Avian species with a high probability of developing aspergillosis (Redig, 1993)

<b>Raptors</b>	<b>Psittacines</b>
Goshawks	Blue-fronted Amazon
Gryfacons	African grey parrot
Immature red-tailed hawks	Mynah bird
Golden eagles	
Rough-legged hawks	<b>Other</b>
Bald eagles	Eider ducks, Trumpeter swans, penguins

#### 2.2. Antifungal Susceptibility Testing

There are over 185 species in the *Aspergillus* genus (Richardson 1998). In avian infections *A. fumigatus* is the most common etiological agent, followed by *A. flavus* and *A. niger* (Bauck 1994). Selection of antimicrobial drugs depends on knowledge of the susceptibility of the suspected pathogen (Prescott 2000), but clinical mycology has lagged behind clinical bacteriology with respect to uniform standardisation of susceptibility testing. *In vitro* antifungal activity does not correlate well with *in vivo* efficacy and without readily available susceptibility tests in veterinary medicine most therapeutic choices are empirically based (Papich *et al.*, 2001). Resistance following antifungal drug use is recognised (Graybill *et al.* 1998). Table 3 presents the activity (MIC) of antifungal agents against fungi &

Table 3. Activity (MIC µg/ml) of selected systemic antifungal agents against selected fungi (Prescott, 2000)

Organism	Amp B	Flucytosine	Ketoconazole	Fluconazole	Itraconazole
<i>Aspergillus fumigatus</i>	1	>64	16	>64	0.25
<i>A. flavus</i>	1	>64	8	>64	0.25
<i>Candida albicans</i>	0.5	0.13	0.13	0.25	0.03
<i>Histoplasma capsulatum</i>	0.25	-	0.25	2	0.06

**Table 4.** Summary of *Aspergillus sp.* susceptibility and routes of administration of commercially available antifungal agents.

Antifungal Drug	Aspergilliosis susceptibility	Route of administration
Griseofulvin	No	p.o.
Flucytosine	Majority of strains resistant**	p.o.
Amphotericin B	Yes, little resistance	i.v., intrathecal <sup>a</sup> , nebulisation
Clotrimazole	Yes	Topical
Ketoconazole	Many strains resistant	p.o.
Enilconazole	Yes, little resistance	Topical, nebulisation
Itraconazole	Yes	p.o. (capsules, suspension)
Fluconazole	Variably effective	i.v., p.o. (tablets, suspension)
Terbinafine	Yes	p.o. (tablets), topical

<sup>a</sup>through the theca of the spinal cord into the subarachnoid space

\*\* synergises with amphotericin B

Table 4 summarises the susceptibility of *Aspergillus sp.* to available antifungal drugs.

### 3. Antifungal Agents Effective Against *Aspergillus sp.* Amphotericin B

**Mechanism of action:** Amphotericin B is a fungicidal polyene antibiotic. It binds ergosterol in the fungal cell membrane making it more permeable, leading to electrolyte leakage and cell death.

**Spectrum of activity:** Organisms with MIC < 1 mg/ml are considered susceptible (Prescott 2000). There is good correlation between MIC values & clinical response (Papich *et al*, 2001). *Aspergillus sp.* are the most frequently reported resistant fungi (Prescott 2000).

**Chemical properties and pharmacokinetics:** Its pharmacokinetics have been poorly studied in veterinary species. It is poorly absorbed from the GIT so must be given i.v., i.o. or i.t. Absorption from the lungs following aerosol administration is poor and this route is used to treat pulmonary aspergillosis (Prescott 2000).

**Adverse effects:** The most important clinical toxicosis is nephrotoxicity (Prescott 2000). It is dose-related and is seen clinically as increases in BUN and creatinine in mammals. Dosing every other day, electrolyte loading and slow infusion of amphotericin B decrease the severity and rate of development of renal toxicity. Other adverse effects include phlebitis, fever, nausea, vomiting and hypokalemia with resulting cardiac arrhythmias (Prescott 2000). Measures to prevent vomiting include giving antiemetic drugs before infusion.

**Clinical protocols:** Dosage regimens are presented in Table 5. Treatment protocols should include pre-treatment with sodium chloride and it should be infused at a slow rate. It can be mixed with 5% dextrose solution during infusion. Administration of amphotericin B in 0.45% saline with 0.5% dextrose s.c. in dogs/cats is a way of administering large quantities of amphotericin B without producing the marked azotemia associated with i.v. injection (Malik 1996).

**New formulations:** New less toxic lipid-based formulations have been used in humans (Walsh *et al* 2001). These drugs can be given at higher doses with less toxicity. In humans daily doses of the lipid-based formulations are 3-5 mg/kg daily, whereas the dose of the conventional form are 0.5-1 mg/kg every 48 hours (Prescott 2000).

#### Flucytosine

**Mechanism of action:** Flucytosine is a fluorinated pyrimidine which is deaminated to 5-fluorouracil and incorporat-

ed into mRNA in the fungal cell, producing faulty proteins.

**Spectrum of activity:** It has a narrow spectrum of activity and few *Aspergillus sp.* strains are susceptible. Strains with MIC < 16mg/ml are susceptible (Prescott 2000). Two thirds of fungal isolates change from susceptible to resistant during treatment so flucytosine should only be used in combination with other antifungal agents (Papich *et al* 2001). Combination with amphotericin B is synergistic because amphotericin B increases fungal permeability to flucytosine (Orosz & Frazier 1995).

**Clinical protocols:** Dosage regimens are presented in Table 5 and only occasional side-effects are reported (Papich *et al* 2001).

#### The Azole Antifungal Drugs

These are broad-spectrum antifungal drugs that exert their antifungal mechanism on the cell membrane of the fungus by inhibiting synthesis of the sterol of the fungal cell membrane. They are generally fungistatic at concentrations achieved clinically and are slower acting than the polyenes.

#### Enilconazole

This drug has excellent antifungal activity. It has a residual effect after topical application and is used to treat topical dermatophyte infections. Enilconazole is the treatment of choice for nasal aspergillosis in dogs & local infusion is used in the treatment of guttural pouch mycosis in the horse (Prescott 2000). It can be given topically or nebulised and the Clinafarm-EC formulation is used in the environmental decontamination of poultry facilities (Table 5) and equipment to prevent aspergillosis.

#### Clotrimazole

This azole is inhibitory to *Aspergillus sp.* and concentrations above 10mg/ml are fungicidal (Prescott 2000). It is available as a topical product, can be nebulised and few strains of fungi are resistant (Prescott 2000).

#### Itraconazole

**Spectrum of activity:** Itraconazole is the drug of choice to treat aspergillosis infections (Papich *et al* 2001). Organisms with MIC < 0.12 mg/ml are susceptible, those with MIC 0.25-0.5 mg/ml are susceptible depending on dose and those with MIC > 1 mg/ml are resistant (Prescott 2000).

**Pharmacokinetics:** Absorption is increased in an acidic environment and when taken with meals. Bioavailability increases from 40% after fasting to 99.8% when given with food (Papich *et al* 2001). It is extensively distributed throughout the body. It is hepatometabolised and mainly eliminated in the bile. Therapeutically active concentrations are maintained longer in tissues than in plasma and the drug can be detected in vaginal epithelium and skin for 4 days and 4 weeks respectively (Papich *et al* 2001).

**Clinical use:** It is used to treat superficial and systemic infections and to treat and prevent aspergillosis in birds

Table 5. Dosage protocols for antifungal agents in birds.

Drug	Species	Route	Dose	Source
<b>Birds</b>				
Amphotericin B (Fungizone)	Raptors Psittacines	i.v.	1.5mg/kg bid for 3-5 days given with 10-15 ml/kg of saline	Redig, 1993a; Biasia & Giovardi, 2001
Amphotericin B (Fungizone)	Raptors Psittacines	i.t.	1 mg/kg lid/bid diluted in saline and given via the glottis	Redig, 1993a; Biasia & Giovardi, 2001
Amphotericin B (Fungizone)	Psittacines	topical	0.05 mg/kg diluted in saline used as surgical irrigation	Biasia & Giovardi, 2001
Amphotericin B (Fungizone)	Psittacines	nebulis e	1 mg/ml saline and nebulised tid for 15 minutes	Ritchie & Harrison, 1994
Flucytosine (Ancobon)	Raptors	p.o.	40-50 mg/kg tid. Always combine with Amphotericin B	Redig, 1993a
Flucytosine (Ancobon)	Psittacines	p.o.	20-50 mg/kg bid. Always combine with Amphotericin B	Ritchie & Harrison, 1994
Clotrimazole	Raptors	Topical nebulis e	Suspend in polyethylene glycol and nebulise for 45 minutes a day	Crosz & Frazier, 1995
Enilconazole (Imavero)	Raptors	i.t.	Dilute 10% solution 10:1 and give 0.5 ml/kg/day for 7-14 days.	Heidenreich, 1995
Itraconazole (Sporonox)	Birds	p.o.	Treatment or prevention - 20 mg/kg sid for 30 days	Papich et al, 2001
Itraconazole (Sporonox)	Raptors	p.o.	10 mg/kg bid in combination with amphotericin B nebulisation tid for 6 weeks	Forbes et al, 1992
Itraconazole (Sporonox)	Raptors	p.o.	10 mg/kg sid	Jones et al, 2000
Itraconazole (Sporonox)	Psittacines	p.o.	10 mg/kg sid from pharmacokinetic studies	Crosz et al, 1996
Fluconazole (Diflucan)	Birds	p.o.	2-5 mg/kg sid	Ritchie & Harrison, 1994
Terbinafine (Lamisil)	Birds	p.o.	10-15 mg/kg bid	Dalhausen, 2000
Terbinafine (Lamisil)	Birds	nebulis e	1 mg/ml solution bid/tid	Dalhausen, 2000
Enilconazole (Imavero)	Mammal, Raptors	topical	Dilute 10% solution 50:1 to form an emulsion and sponge animal every 3 or 4 days for 4 treatments.	Forbes, 1995; Papich et al, 2001
F10	Raptors Psittacines	Fog or nebulis e	1:250 dilution of F10 superconcentrate fogged or nebulised for 15-30 min/day bid/tid.	Verwoerd, 2001; Bailey & Sullivan, 2001
<b>Environmental Decontamination</b>				
Enilconazole (Cinifam-EC)	Poultry facilities	Fog or spray	Dilute 13.8% solution 1:100 and spray or fog facility.	Papich et al, 2001
F10	Facilities	Fog	1:250 dilution of F10 superconcentrate fogged	Anon, 2001

(Table 5). As oral absorption is pH dependent dosage adjustments are necessary if gastric pH is increased (Papich *et al* 2001). Oral capsules should be given with food, but the oral suspension is better absorbed on an empty stomach. Orosz *et al* (1995) reported higher tissue concentrations when itraconazole is dissolved in acid and gavaged with orange juice in pigeons (*Columba livia*). Treatment of serious infections should be prolonged (>3 months) and relapses occur (Prescott 2000). An i.v. formulation is being developed and is effective against experimental disseminated aspergillosis in guineapigs (Odds 2000).

Adverse effects: Itraconazole is better tolerated than ketoconazole. Hepatic toxicosis occurs in 10% of dogs, while in cats there are dose-related GIT effects of anorexia and vomiting (Papich *et al* 2001). Long-term administration (3 months) to dogs and cats resulted in no side-effects, but maternal toxicity, embryo toxicity and teratogenicity were observed in rats; therefore, its use in pregnant mammals is not recommended (Prescott 2000). Itraconazole is considered to cause anorexia, depression and death in African grey parrots (*Psittacus erithacus*) at doses above 5 mg/kg bid (Birdmed discussion 2002).

#### Other Antifungal Agents Terbinafine

This synthetic allylamine drug is highly fungicidal. Allylamines decreases the fungal synthesis of ergosterol and cause fungal death by disrupting the cell membrane (Prescott 2000). It is active against *Aspergillus sp.* but to

date clinical use has been limited in veterinary medicine, although preliminary nebulisation results are promising (Dalhausen 2000).

#### F10-Disinfectant

F10 is a complete spectrum virucidal, bactericidal, fungicidal & sporicidal, but aldehyde free compound of six synergistic active ingredients (Verwoerd 2001). It has been tested against every significant animal/human pathogen and has outperformed other disinfectants during efficacy testing, over a range of temperatures, in the presence of organic material, at low concentrations, short contact times, without any corrosive effects on infrastructure, metal nozzles or any tissue irritation on workers and animals (Anon 2001). With these characteristics, F10 can be used to disinfect animal environments in their presence, lowering the environmental pathogen challenge significantly, with no side-effects. Using either a nebuliser or a 'smogger' unit, F-10 has been used to treat either individuals or groups of falcons with aspergillosis (Table 4; Bailey and Sullivan, 2001).

#### New Antifungal Agents

Voriconazole & echinocandin are new triazole & lipopeptide drugs respectively which show promise against aspergillosis (Prescott, 2000; Graybill, 2001).

## 4. Therapy

### Combination Therapy

In vitro synergism of 2 antifungal compounds, seen as a 4x reduction in the MIC when given alone is seen when amphotericin B is given with flucytosine (Papich *et al* 2001). Combinations of amphotericin B and azoles have been less successful and both antagonism and synergism have been reported (Bajjoka *et al* 1999). Because of the slow onset of action of azole antifungals it is recommended to use initial amphotericin B therapy of serious systemic fungal infections followed by an azole agent (Papich *et al* 2001). The treatment combinations used at Abu Dhabi Falcon Hospital are presented in Table 6.

Table 6. Treatment regimens used at Abu Dhabi Falcon Hospital in aspergillosis cases (1999-2001).

Clinical case	Treatment (doses in Table 5)
Mild cases (good prognosis)	Nebulise amphotericin B or F10 or enilconazole bid (1 week) Atomise F10 (when nebulising finished for 2-3 weeks) Per os itraconazole bid (4-8 weeks)
Moderate-cases (guarded prognosis)	Nebulise amphotericin B or F10 or enilconazole bid (1 week) Atomise F10 (when nebulising finished for 2-3 weeks) I.V. amphotericin B 5-7 days Per os itraconazole bid (4-8 weeks)

### Surgery

In advanced cases surgical removal of granulomas from the airsacs, or lesions that are blocking the trachea or syringe and direct application of antifungal agents to the lesions are important therapeutic adjuncts in birds (Tully *et al* 2000). Studies in dogs with nasal aspergillosis have shown that while systemic therapy is successful in 50% of cases, systemic therapy and local irrigation of nasal lesions is successful in 90% cases (Clark 1999).

### Supportive Therapy

Supportive therapy including fluids (i.v., s.c., i.o.), tube feeding, antibiotics, antiemetic drugs, immune stimulants and vitamin A supplementation should be given according to the needs of the case. The use of immunosuppressive drugs (steroids) in birds is controversial and should be avoided (Chitty 2001). Indeed cortisone-treated animals (rabbits) are more susceptible to invasive aspergillosis (Khosravi *et al* 1998).

### Immune Stimulation

Interferon  $\gamma$  augments the ability of human leucocytes to damage fungal hyphae *in-vitro* and clinical trials of colony stimulating factor have shown that it can be a useful adjunct to antifungal therapy (Richardson 1998). Immune stimulation may have a role in veterinary species in the future.

## 5. Duration and Response to Treatment

### Duration of Therapy

Antifungal regimens in humans are often extended for 6-12 months (Jones *et al* 2000). For veterinary species treatment duration recommendations vary from 2 to 6 months and one month past the resolution of the disease (Jones *et al* 2000; Tully *et al* 2000).

### Response Rate and Susceptibility to Re-infection

Interestingly, despite numerous treatment protocols (Table 5), little data has been published on the response to treatment so that the efficacy of different regimens can be objectively assessed. Mortality from invasive aspergillosis in immunocompromised humans is high (Patterson *et al* 2000). The average case fatality rate (CFR) of 50 published studies (1995-2000) on the treatment of invasive aspergillosis in humans was 58% (SwuJane 2001). In a survey of aspergillosis cases in a large falcon hospital the CFR for falcons was 37.5% (Table 7). Little has been published on the susceptibility of recovered animals to rechallenge with aspergillosis. Kunkle and Sacco (1999) demonstrated that convalescence from pulmonary aspergillosis in turkeys did not confer protection against rechallenge but instead decreased resistance to subsequent infection. Recrudescence of

**Table 7. Review of 49 Aspergillosis cases seen at Abu Dhabi Falcon Hospital 2000-2001 in Falcons (see Table 6 for treatment regimens)**

Aspergillosis Grade (Redig, 1993a)	Group A Treated and recovered	Group B Treated and died	Group C Euthanased after diagnosis
1	0	2	0
2	0	0	0
3	16	3	0
4	4	7	17
<b>Total</b>	<b>20</b>	<b>17*</b>	<b>17</b>

\*Five cases the owner was advised that the birds carried a poor prognosis and euthanasia was recommended, but the owners insisted on attempting treatment.  
 Case Fatality Rate - 46% (17/37) for all birds receiving treatment  
 Case Fatality Rate - 37.5% (12/32) for birds receiving treatment where the vet considered treatment carried a reasonable chance of success (Groups A and B excluding 5 cases where euthanasia was recommended after diagnosis, but owners insisted on treatment).

aspergillosis infections in falcons that have recovered from aspergillosis is common in the Middle East and it is likely that the CFR of 37.5% is higher as this does not account for recrudescence because of difficulties in case follow-up.

## 6. Prophylaxis, Prevention & Vaccination

### Prophylaxis

Prophylactic antifungal therapy is recommended for high risk humans and itraconazole is considered the most effective antifungal agent at preventing aspergillosis (Kibbler 1999). Likewise, prophylactic itraconazole (Table 5) is recommended for captive-held birds undergoing a change in management, especially high risk species (Table 1) and during times of stress such as trauma (Redig 1993a) or as part of the management of oiled susceptible seabirds. *Aspergillus spp.* can colonise damaged airways (Richardson 1998), so probably prophylactic antifungal medication should be considered as part of the therapy of many respiratory tract infections (e.g. *Serratospiculiasis*) of susceptible species. Daily fogging with F10 of susceptible birds is used to lower the environmental load of respiratory pathogens such as *Aspergillus sp.* in the Middle East.

### Vaccination

Vaccination has been proposed as a means of preventing aspergillosis. A heat-killed vaccine reduced mortality of aspergillosis in waterfowl (Yearout 1988) and a germinated conidia vaccine reduced turkey poult mortality (Richard *et al* 1984). Recently, Graczyk *et al* (1997) demonstrated that i.m. immunisation of Cape shelducks (*Tadorna cana*) with *Aspergillus spp.* mycelial phase cultures provided protection against experimental challenge. However, despite these reports no aspergillosis vaccinations are commercially available for any veterinary species.

### Prevention

Recommendations to prevent aspergillosis in captive avian collections are presented in Table 8.

**Table 8. Recommendations to prevent aspergillosis in captive avian collections (Hary & Cooper, 1970; Wobeser, 1981; Richard, 1991; Redig, 1993a; Dykstra *et al.* 1997)**

High standards of hygiene to maintain a healthy environment (reduce exposure to mouldy vegetation in litter and feed)
Environmental screening of avicultural facilities
Increase ventilation within facility
Spraying litter, facilities with disinfectant
Fogging facilities in the presence of live animals/birds with F10 disinfectant
Reduce stress (especially newly captured birds)
Serological screening programme (in raptors at the University of Minnesota)
Egg-transmitted aspergillosis in poultry has been controlled by treating the eggs with phenylmercuric dinaphthylmethane-disulfonate.

## 7. Conclusions

Aspergillosis is a manageable and treatable disease if detected early, but advanced cases are medically challenging. Well-designed studies to determine the correlation between the susceptibility of avian isolates and the clinical responses to antifungal drugs are needed so that therapeutic regimens can be optimised.

## 8. Glossary

bid = twice a day; i.o. = intraosseus; p.o. = per os; CFR = case fatality rate, i.t. = intratracheal injection; s.c. = subcutaneous; GIT = gastro-intestinal tract; i.v. =, intravenous injection; sid = once a day; i.m. = intramuscular injection; MIC = minimum inhibitory concentration; tid = three times a day

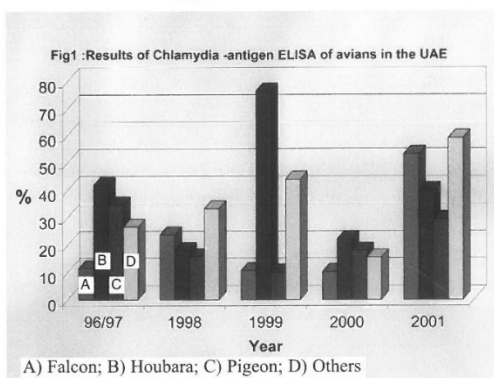
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# An update on the prevalence of Chlamydia infections in avians in the U.A.E.

U. Wernery, W. F. D'Mello and R. Zachariah  
Central Veterinary Research Laboratory, P.O. Box 597,  
Dubai, U.A.E.

Over the last years we have twice reported about *Chlamydia*-infections in avians (Zachariah and Wernery, 1997 and 1998) in the U.A.E. The reason to update the results, is a sharp increase of positive cases especially in falcons in 2001 (Table 1 and Figure 1).



Chlamydiosis and *Chlamydia*-infections in falcons and other avian species are a persistent problem in the U.A.E., but it is so far not clear why the increase has suddenly occurred in 2001. For the diagnosis of an infection, we use the *Chlamydia*-antigen ELISA from DAKO, UK (IDEIA, Code No. K601).

Swabs are taken from the pharynx and are immediately placed into *Chlamydia* transport medium. They are then sent to CVRL for testing. Many of the positive birds do not show any clinical signs. However, they have a *Chlamydia*-infection and may suffer from Chlamydiosis when under stress.

The ag-ELISA does not differentiate between the different *Chlamydia* species as the antigen used in the test shares a common antigen with all the different species. The test utilizes a monoclonal antibody to detect a genus-specific

chlamydial antigen. Further work should now concentrate on the identification of *Chlamydia* species in U.A.E. avians. For this purpose a tissue culture laboratory should be established. The new *Chlamydia* classification is seen in Table 2.

Table 2.

	Previous classification	Revised classification	
Order	Chlamydiales	Chlamydiales	
Family	Chlamydiaceae	Chlamydiaceae	
Genus	<i>Chlamydia</i>	<i>Chlamydia</i>	<i>Chlamydrophila</i>
Species	<i>C. trachomatis</i>	<i>C. trachomatis</i>	
		<i>C. muridarum</i>	
		<i>C. suis</i>	
	<i>C. pneumoniae</i>		<i>C. pneumoniae</i>
	<i>C. psittaci</i>		<i>C. psittaci</i>
			<i>C. abortus</i>
			<i>C. felis</i>
			<i>C. caviae</i>
	<i>C. pecorum</i>		<i>C. pecorum</i>

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Table 1: Results of Chlamydia -antigen ELISA in the avians in the UAE

Species	96/97			1998			1999			2000			2001		
	Total no:	+	%	Total no:	+	%	Total no:	+	%	Total no:	+	%	Total no:	+	%
Falcon	874	101	11.5	508	119	23.8	1004	108	10.7	577	59	10.2	218	116	53.2
Houbara	155	65	41.9	116	21	18.0	51	39	76.5	18	4	22.2	5	2	40.0
Pigeon	23	8	34.7	19	3	15.7	10	1	10.0	67	12	17.9	17	5	29.4
Others*	30	8	26.6	10	3	33.3	18	8	44.0	26	4	15.4	39	23	59



## What's new in the literature ?

**Barton, N.W.H., Fox, N.C., Surai, P.F. and Speake B.K. (2002) Vitamins E and A, carotenoids and fatty acids of the raptor egg yolk. *Journal of Raptor Research* 36 (1) 33-38.**

A captive population of falcons was fed a diet containing a known quantity of vitamin A (retinol) and vitamin E (α-tocopherol) for six weeks prior to and during egg-laying. Infertile eggs were analysed for vitamin A, vitamin E, carotenoid and fatty acid composition. Mean, daily vitamin intake was 29mg Vit E (35IU) and 1157mg Vit A (3363IU). Adjusted mean egg yolk content for infertile, unincubated eggs was 314 mg/g α-tocopherol and 3.06 mg/g Vit A. A distinctive feature of the raptor egg yolk is a very high proportion of arachidonic acid which is probably a reflection of their carnivorous diet. A small number of plasma samples were also available from egg-laying falcons. Mean, plasma vitamin E was 32.2 mg/ml and plasma vitamin A 1.02 mg/ml.

**Casado, E., Balbontin, J., Ferrer, M. (2002) Plasma chemistry in booted eagle (*Hieraetus pennatus*) during breeding season. *Comparative Biochemistry and Physiology. A, Molecular & Integrative Physiology*, 131, No.2, pp.233-241**

Most studies that have examined raptor plasma chemistry have been conducted on birds living in captivity. In this study, we describe typical plasma chemistry values indicators of body condition in free-living Booted Eagles, *Hieraetus pennatus*, from Donana National Park (Spain). A total of 143 young, 55 adults and one bird of unknown age were caught, banded and sampled between 1996 and 2000. Values were compared with those of other raptors. Mean concentrations of creatinine, uric acid and urea were lower in adults than in nestlings, while glucose, DAT and AAT were lower in nestlings than in adults. Interactions of age/sex affected plasma mean levels of creatine kinase, glucose, AAT, uric acid and urea. Adult females showed significantly lower levels of creatine kinase, uric acid and urea than adult males and nestlings. Adult males had significantly higher levels of AAT than the other groups. The lowest levels of glucose and the highest levels of uric acid were found in nestling females. We think the differences in blood parameters can be explained by differences in size of species, of individuals (because of both body condition and sexual dimorphism) and diet.

**Dawson, R. D., Bortolotti, G. R. (2001) Sex-specific associations between reproductive output and hematozoan parasites of American kestrels. *Oecologia*, 126, No.2, pp.193-200**

Parasites have the potential to decrease reproductive output of hosts by competing for nutrients or forcing hosts to invest in immune function. Conversely, reproductive output may affect parasite loads if hosts allocate resources to reproduction such that allocation to immune function is compromised. Both hypotheses implicitly have a temporal component. Parasites (*Haemoproteus spp.*) were sampled

both before and after egg laying to examine the relationship between reproductive output (indexed using a combined measure of clutch size, egg volume and initiation date) and blood parasite loads of American kestrels (*Falco sparverius*). Parasite loads measured prior to egg laying had no adverse effects on subsequent reproductive output. Females that previously had large reproductive outputs subsequently had lower parasite intensities than those whose outputs were smaller, suggesting that females were capable of allocating energy to both forming clutches and reducing parasite loads. Because male kestrels provide most of their mate's energetic needs before, during and after egg laying, mate choice by females may have consequences for their parasite loads. Females choosing high-quality mates may not only have increased reproductive output, but may also obtain sufficient resources from their mates to enable them to reduce their parasite burdens. Males whose mates had large reproductive outputs were more likely to subsequently be parasitized and have more intense infections. For individual males sampled both before and after egg laying, those whose mates had larger reproductive outputs were also more likely to become parasitized, or remain parasitized, between sampling periods. Increased parasite loads of males may be one mechanism by which the costs of reproduction are paid.

**Dawson, R. D. and Bortolotti, G. R. (2000) Effects of hematozoan parasites on condition and return rates of American kestrels. *Auk* 117, No.2, pp.373-380**

The relationship between blood parasites and body condition of American kestrels (*Falco sparverius*) was evaluated during the breeding season. Females that were infected with at least one species of parasite were in poorer condition than those without parasites during incubation but not prior to egg laying. It is suggested that the relationship between parasitism and condition was masked before laying because of large increases in body mass of females during egg formation. Reduced condition of males during incubation also was associated with higher intensity of infections by *Haemoproteus* in one of 2 years. The negative association between condition and intensity of infection suggests that blood parasites impose costs on kestrels owing to competition for nutrients or allocation of energy by hosts to immune function or tissue repair. Alternatively, kestrels in poor condition may be more likely to have relapses of chronic infections, or they may be less able to control new infections because of resource limitations. In contrast to results during incubation, during the prelaying period the prevalence of parasites tended to be higher, and in one year infections were more intense, among males in good condition. One possible explanation is that body condition of males during courtship is an important determinant of the quality of mate they are able to obtain, and males may be accumulating body reserves at the expense of decreased immune function. Return rates of female kestrels to the study area declined as the intensity of their *Haemoproteus* infections increased, suggesting that blood parasitism is associated with reduced survival or increased

dispersal probability.

**Eastham, C.P., Nicholls M.K. and Fox, N.C. (2002) Morphological Variation of the Saker (*Falco cherrug*) and the implications for conservation. Biodiversity and Conservation 11: 305-325.**

This study assesses the external morphology of the saker which is highly variable. Although most variation was between individuals within regions, the remaining variation in size and plumage characters may be described as a gradual cline between small, dorsally uniform brown sakers from western lowland regions (Kazakhstan and south west Russia) to large grey coloured sakers from eastern highland regions (south east Russia, Mongolia and China). Between these extremes exists a plethora of highly variable and contiguous populations.

**Krone, O., Priemer, J., Streich, J., Sommer, P., Langgemach, T., Lessow, O. (2001) Haemosporida of birds of prey and owls from Germany. Acta Protozoologica 40, No.4, pp.281-289**

1149 free-living birds of prey from Germany were examined for blood parasites. The prevalence of infection was 11% (adult birds 18%, immature birds 16%, and nestlings 4%). Among the Falconiformes and Strigiformes, 11% of 976 and 13% of 173 birds, respectively, were infected. Of 17 falconiform species, 9 were infected with blood parasites whereas the Eurasian buzzard (*Buteo buteo*) had the highest prevalence for haematzoa; i.e. *Leucocytozoon toddi* (31%), the highest prevalence (25%) for *Haemoproteus* sp. was found in the hobby (*Falco subbuteo*). Eight species of owls were examined for blood parasites; the tawny owl (*Strix aluco*) had the highest prevalence with *Haemoproteus syrnii* (22%). In one pygmy owl (*Glaucidium passerinum*) examined, *Trypanosoma avium* and *Plasmodium fallax* were detected. The white-tailed sea eagle (*Haliaeetus albicilla*) was found to be a host of *L. toddi* for the first time. Differences in the prevalence of blood parasites were found in the seasons and age classes of the birds.

**Lierz, M., Gobel, T., Schuster, R. (2002) Review and investigations on parasites in birds of prey and owls found injured or debilitated. Berliner und Munchener Tierarztliche Wochenschrift 115, No.1/2, pp.43-52**

In the present paper, a general overview on parasites in birds of prey and owls is given. This part is followed by a study investigating the prevalences and species of parasites in free-ranging birds of prey and owls in Berlin and Brandenburg State, Germany. In a period of one year (between August 15, 1996 and August 15, 1997), 84 birds of prey and owls of the following species were examined for the presence of endo- and ectoparasites: Common Buzzard (*Buteo buteo*; n=32), Kestrel (*Falco tinnunculus*; n=20), Sparrowhawk (*Accipiter nisus*; n=9), Goshawk (*Accipiter gentilis*; n=8), Black Kite (*Milvus migrans*; n=4), Peregrine Falcon (*Falco peregrinus*; n=3), Marsh Harrier (*Circus aeruginosus*; n=1), White-tailed-Sea Eagle (*Haliaeetus albicilla*; n=1), Tawny Owl (*Strix aluco*; n=4), Long-eared Owl (*Asio otus*; n=1) and Barn Owl (*Tyto alba*; n=1). In 97.6% of the cases, ectoparasites (feather

mites and hippoboscoid flies) were found. It was observed that 93.3% of eyasses were positive for hippoboscoid flies. *Trichomonas* was detected in 28.6% of all birds of prey and owls examined. A prevalence of 100% was established in the Sparrow Hawks as well as Peregrine Falcons. *Leucocytozoon* sp. and *Haemoproteus* sp. as blood parasites were found in 26.9% of the birds in total. Common Buzzards showed the highest prevalence (44.8%). A total of 58.3% of birds examined were positive for endoparasites. Flukes were found in 16.7%, tapeworms in 14.3%, roundworms in 48.8% and acanthocephales in 2.4% of the cases. Interestingly, *Tylodelphis clavata* (in a Common Buzzard) and *Hovorkonema variegatum* (in a Goshawk) were found for the first time in raptors. The results of this study underline the importance of a parasitological examination in the process of raptor rehabilitation.

**Manvell, R. J., McKinney, P., Wernery, U., Frost, K. (2000) Isolation of a highly pathogenic influenza A virus of subtype H7N3 from a peregrine falcon (*Falco peregrinus*). Avian Pathology 29, No.6, pp.635-637**

A peregrine falcon (*Falco peregrinus*) was presented to the Al Safa Falcon Clinic in Dubai, United Arab Emirates unable to stand [date not given]. Four hours after hospitalization, the bird died despite supportive care and calcium disodiumedetate treatment. The falcon had been on a hunting trip to Syria with its owner of 2 years, prior to its death. The carcass was submitted to the Central Veterinary Research Laboratory in Dubai where it was subjected to postmortem examination. Investigations resulted in the isolation of an influenza A virus subtype H7N3, which proved to be highly pathogenic for chickens.

**Parga, M. L., Pendl, H., Forbes, N. A. (2001) The effect of transport on hematologic parameters in trained and untrained Harris's hawks (*Parabuteo unicinctus*) and peregrine falcons (*Falco peregrinus*). Journal of Avian Medicine and Surgery 115, No.3, pp.162-169**

Birds of prey are found increasingly in captive situations. When sick, these animals show only very subtle signs of disease, making the detection of disease difficult. For this reason, the analysis of haematological parameters is a very useful technique for the avian veterinarian. However, birds are most frequently transported to the veterinary clinic for a health check-up. This study investigates the effects of transport-related stress on heterophil and lymphocyte morphology and haematological parameters of peregrine falcons (*Falco peregrinus*) and Harris's hawks (*Parabuteo unicinctus*). Twelve birds of each species from the UK, all adults, mixed males and females, were analysed. Each group of 12 was comprised of 6 trained (accustomed to being transported) and 6 untrained birds. Samples of blood were taken from all birds in their place of origin and again 1 week later after 1 hour (35 km) of transport. Both samples were taken in similar conditions (eg, time of day, duration after feeding, environmental temperature, sample handling) so that any variation would be caused only by transport-related factors. Both untrained groups showed a significant ( $P < 0.05$ ) increase in the heterophil/lymphocyte ratio (H/L). Whereas untrained peregrines showed no other significant change, untrained Harris's hawks had a signifi-

cant leukopenia lymphopaenia and eosinopaenia. Trained Harris's hawks showed a significant monocytosis, whereas trained peregrines showed no significant change. Transport had no apparent influence on heterophil or lymphocyte morphology. Although the difference between pre- and posttransport was significant in some parameters, all values in the 4 groups remained within the reference ranges for the species. Therefore, we can conclude that 1-hour transport for trained or untrained members of these 2 species to a clinic need not be factor that requires the clinician's consideration when interpreting a haematological sample. However, the 2 species reacted differently to transport. Further studies on other species are suggested. The H/L ratio is proposed as the most sensitive measure of stress response in the blood picture of raptors and possible uses are suggested.

**Samour, J. H., Naldo, J. L., John, S. K. (2001) Staining characteristics of the eosinophil in the saker falcon. Exotic DVM 3, No.4, p.10**

The differentiation of eosinophils is one of the most difficult tasks encountered during hematology analysis in avian species, but in particular in birds of prey. In addition, the eosinophils and heterophils share many morphological characteristics making differentiation between them challenging. These difficulties can be overcome by the adequate staining of these cells and by becoming aware of the relevant differences of the normal morphology of heterophils and eosinophils in the different avian species. During routine hematology analysis of samples from saker falcons it was found that the staining of granules of eosinophils using May Grünwald Giemsa stain was not satisfactory. After some trials involving different stains, timings, concentrations and buffers, the best results were obtained using a modified Wright's-Giemsa stain. The modification to the Wright's-Giemsa stain consisted of adding 5.0ml of glycerol and including step number four to the staining procedure. Diff-Quick or similar stains are not recommended for routine hematology analysis because these techniques tend to rupture the granules of the granulocytes. The use of the modified Wright's-Giemsa stain as described can probably be applied to other avian species in which cell differentiation has proved difficult.

**Wernery, U., Joseph, S., Kinne, J. (2001) An attenuated herpes vaccine may protect Gyr hybrids from fatal inclusion body hepatitis. A preliminary report. Journal of Veterinary Medicine. Series B, 48, No.10, pp.727-732**

Four Gyrfalcon hybrids were used for this falcon herpes vaccine trial. Three falcons were given 1 ml of an attenuated falcon herpesvirus vaccine (DuFaHe) subcutaneously twice within 14 days, whereas the fourth falcon was used as a control. 18 days after the booster vaccination, all 4 Gyr hybrids were intranasally and ocularly challenged with a virulent low-passage falcon herpesvirus. The control falcon died 9 days after the challenge, with typical lesions of herpesvirus inclusion body hepatitis. The

3 vaccinated falcons seroconverted and did not show any symptoms. Following the challenge, their antibody titres to falcon herpesvirus increased. No herpesvirus was isolated from any of the cloacal swabs taken during this experiment, indicating that there was no danger for any other birds from DuFaHe. This experiment shows that falcons can be protected from herpesvirus infection by an attenuated herpesvirus vaccine. However, it should be stressed that only 4 falcons were used for this experiment.

## Conference announcement

**6th World Conference  
on Birds of Prey and Owls**

**Budapest  
HUNGARY  
18-25 May 2003**

**Deadline for abstract submissions 1 March 2003**

For further information contact:  
World Working Group on Birds of Prey  
P.O. Box 52, Towcester, NN12 7ZW, England

Tel/Fax +44 1604 862331  
Email: WWGBP@aol.com or  
robin.chancellor@virgin.net



Gyr/Saker

