PAPER

Reducing visual stimulations in European hares (*Lepus europaeus* Pallas) captured for translocation

Gisella Paci,[1] Marco Ferretti,[2] Marco Bagliacca[3]

[1]Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti, Università di Pisa, Italy
[2]Servizio Agricoltura, Patrimonio Naturale ed Ittio-faunistico Gestione Aree Protette, Ufficio della Provincia di Pistoia, Italy
[3]Dipartimento di Scienze Fisiologiche, Università di Pisa, Italy

Abstract

Stress may be an important variable affecting the survival of the captured hares, for this reason the Authors studied the effect of the use of a blindfolding hood applied to the hares immediately after net trapping. A total of 119 hares were captured by coursing with 3-4 dogs (greyhounds or lurchers) in no hunting areas of Tuscany. Immediately after net trapping 38 hares were blindfolded with a hood and 81 were normally handled to the wooden darkened capture-boxes without blinding their eyes (control). From all the hares a sample of blood was collected within 1-2 min by the auricular vein. Blood samples were analyzed for haemocromocytometry and metabolic profile determination. Data were analyzed by ANOVA. Sub-clinically stressed hares were discriminated from non-stressed hares by the use of the discriminant function based on CK, AST and glucose and the differences were tested by χ^2_c. Results showed that body temperature, heart and respiratory rates, glucose, cholesterol, CK, AST, ALT, BUN, total protein and some haemocromo-cytometric parameters were significantly higher in captured hares without blindfold. The incidence of subclinically stressed hares was 26% in the hares equipped with the blindfold and 81% in the hares without blindfold (χ^2_c = 32.98, P<0.0001) (presence of subclinical stress discriminated by the use of a discriminant function based on CPK, AST and glucose). For this reason the procedures designed for the translocation of the hares could take advantage by the use of a blindfold which should be applied to the hares immediately after their trapping.

Introduction

The brown hare (*Lepus europaeus*) is common throughout Europe except in the northern most part where the mountain hare (*Lepus timidus*) occurs. The brown hare is an important game animal. To restore the right population density in low densities free hunting territories it is often necessary to introduce wild or reared foreign subjects.

A valuable technique for restocking, inside low densities free hunting territories, is the release of brown hares captured in high-density protected areas (Ferretti *et al.*, 2010) and/or in large enclosures (Santilli *et al.*, 2004). Capture for translocation (transport and release in different areas, presenting more or less similar habitats) may cause direct death or stress that can play a role in the development of future complications in the released hares. The stress may be light, with no physiological consequences; it may be heavy and cause physiological alterations, or it may be extremely heavy and cause also cell deaths (Paci *et al.*, 2006). In any case stress which alters the normal physiology of the animals can reduce the immune response (Spraker, 1993; Ranucci *et al*., 1996; Williams and Thorne, 1996; Diverio *et al*., 1998; Montané *et al*., 2003; Paci *et al.*, 2006; Paci et al., 2011). Stress can be suppressive (if prolonged) or stimulatory (if transient acute) on the immune response (Amadori *et al.*, 2009). The capture could be considered as a transient acute stress which might be associated with a better immune response and, consequently, as nature's adjuvant in natural condition. Symptoms are generally not obvious at the time of capture but myopathy should be of concern. Death may occur almost immediately or up to 1 month after handling (Bartsch *et al*. 1977; Chalmers and Barrett, 1982). For this reason, we performed a study to evaluate the effect of the use of a blindfold to reduce stress during the capture operations of the hares.

Materials and methods

The samples were collected from brown hares that were caught in Tuscany (central Italy) during 2007-2009. A total of 119 hares were sampled in 14 different non-hunting high-density areas where hares are usually captured for subsequent release in free hunting low-density areas (Paci and Bagliacca, 2003). The hares were captured by coursing with 3-4 dogs (greyhounds or lurches). Different teams of hunters released the dogs to find and drive into trammel nets any hare that was seen running. The team of hunters was composed of game and wildlife officers, volunteers, and game wardens. The hares ran for different distances and varying periods of time in relation to the size of the area.

The operators positioned near the trammel nets were randomly equipped or not with a blindfold fitted for the hares (Figure 1). A total of 38 hares were equipped with the blindfolds just in the net and 81 hares were removed from the net without covering their eyes. After capture, all the hares were located and remained inside darkened, wooden capture-boxes for a different period of time (10' trough 30') before the blood drawing. For sanitary monitoring all the hares were then removed from the boxes, physically restrained and blood was always collected within 1-2 min (blood was drawn from the auricular vein using disposable syringes

Corresponding author: Prof. Gisella Paci, Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti, Facoltà di Medicina Veterinaria, Università di Pisa, viale Piagge, 2, 56124 Pisa, Italy.
Tel. +39.050.2216903 – Fax: +39.050.2216901.
E-mail: gpaci@vet.unipi.it

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and 0.5×16 mm needles). Body temperature (by digital thermometer introduced in the rectum), as well as heart (mediated auscultation by phonendoscope) and respiratory rate (by visual observation), were measured in each hare.

Blood samples were divided into two subsamples:

- The first sub-samples (0.5-1 ml EDTA-plasma) were analyzed within 24 h by automatic complete blood counter (Seac Heco Vet C, Calenzano, FI, Italy). The following analyses were performed: white blood cell (WBC); red blood cell (RBC); haemoglobin (Hgb); haematocrit (Hct); mean blood cell volume (MCV), mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); red blood cell distribution width (RDW); platelets (Plt); mean platelet volume (MPV); platelet haematocrit (Pct); platelet distribution width (PDW).
- The second sub samples (0.5-1 mL of Li-heparin plasma,) were frozen at -20°C and the following analyses were performed on plasma with AU400 Olympus using Olympus Diagnostics Kits: Glucose (GLU): colorimetric determination with oxidase-peroxidase (Sclavo, Siena, Italy); Cholesterol: enzymatic colorimetric determination (Sclavo); Triglicerides: enzymatic colorimetric determination (Poli Diagnostici, Italy); Creatine kinase or creatine phosphokinase (CK): kinetic colorimetric method (Sclavo); ALanine amino Transferase (ALT): kinetic UV test IFCC (Sclavo); ASpartate amino Transferase (AST): kinetic UV test IFCC (Sclavo); Non-Esterifies Fatty Acids (NEFA): enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany); Blood Urea Nitrogen (BUN): enzymatic colorimetric method (Intermedical, Grassobbio, BG, Italy); Total protein: colorimetric, Endpoint biureto-method (Futurlab, Limena, PD, Italy); Albumins: colorimetric method (SEAC, Radim Group, Calenzano, FI, Italy); Globulins: electrophoresis of serum protein carried out by Microtech 648R (Interlab, Roma, Italy); Cortisol by radioimmunoassay (SEAC). The relationship between the blood biochemistry of the hares equipped with the blindfold *vs* the hares of the control group was analyzed by one-way ANOVA and confirmed by non-parametric test (Wilcoxon rank sums test) in the bordering values. Sub-clinically stressed hares were discriminated from non-stressed hares by the use of the discriminant function based on CK, AST and Glucose determined by Paci *et al.* (2006). The different incidence of sub clinical stress within the hares equipped with the blindfold *vs* the hares of the control group was tested by χ^2 (SAS, 2009).

Results and discussion

Results showed that temperature, respiratory rate and hearth rate were significantly increased in the hares without blindfold compared to the hares equipped with blindfold (Table 1). Both hearth rates however were within the limit observed for healthy hares (Nicpo *et al.*, 2007; Paci *et al.*, 2007; Noszczyk-Nowak *et al.,* 2009). In particular, the highest level of temperature, respiratory and hearth rate in hares without blindfold can be considered as the response to difficult situations perceived by individuals (Broom and Johnson, 1993; Diverio *et al*., 1996): the captured animals without blindfold perceive the constriction to be a dangerous situation more than the hares with blindfold.

Also the haematic parameters determined on Li-plasma were comparable to those observed in previous observations (Paci *et. al.*, 2007; Massányi *et al.*, 2009). However, even if within physiological values, significant differ-

Figure 1. The hood used to blindfold the hares.

Table 1. Physiological and haematic parameters of the hares after capture.

	Hares with blindfold			Hares without blindfold			
	N	Mean	SE	N	Mean	SE	Prob.>F
Temperature, °C	23	36.98	0.0864	83	37.93	0.164	<0.0001**
Respiratory rate, n/min	23	72	2.19	83	86	4.17	0.0032**
Hearth rate, n/min	23	109	4.39	83	133	8.35	0.0147*
Glucose, mg/dL	38	161	7.1	64	198	9.2	0.0019**
Cholesterol, mg/dL	38	137.0	18.9	62	172	14.8	<0.01**
Triglycerides, mg/dL	38	67.1	8.64	64	83.0	6.66	0.1501
CK, U/L	38	2220	669	81	4476	458	0.0063**
ALT (or GPT), U/L	38	49.6	4.16	81	63.9	2.85	0.0052**
AST (or GOT), U/L	36	123	18.3	81	171	12.2	0.0303*
NEFA, mg/L	38	43.2	1.00	65	38.2	1.30	0.0031**
BUN, mg/dL	38	44.5	2.31	81	53.9	1.58	0.001**
Total protein, g/dL	38	5.70	0.171	64	6.30	0.132	0.0063**
Albumins, g/dL	38	4.26	0.128	64	3.74	0.098	0.0016**
Globulins, g/dL	36	1.29	0.137	81	2.01	0.091	<0.0001**
A/G ratio	22	4.45	0.759	15	3.88	0.919	0.6342
Cortisol, μg/dL	38	12.7	1.35	67	12.4	1.01	0.8749

SE, standard error; CK, creatine kinase; ALT, alanine amino transferase; AST, aspartate amino transferase; NEFA, non-esterifies fatty acids; BUN, blood urea nitrogen. *P<0.05; **P<0.01.

ences were observed between the hares equipped with the blindfold and the hares captured and kept with the naked eyes for glucose, cholesterol, CK, ALT (or GPT), AST (or GOT), NEFA, BUN, total protein, albumins and globulins. The captured hares without blindfold presented higher glucose value and lower level of NEFA than hares with blindfold (198 mg/dL *vs* 161 mg/dL, P<0.01; 38.2 mg/dL *vs* 43.2 mg/dL, respectively), this can be explained by the hyperglycaemic effect of catecholamines and glucocorticoids released during stress involved in the hares captured without blindfold (Spraker, 1993).

Cholesterol concentration resulted higher in the hares without blindfold (172 mg/dL *vs* 137 mg/dL, P<0.01): even if the level of cholesterol can vary with the diet and the time of year also other Authors have found higher concentrations of cholesterol in captured animals and attributed higher cholesterol levels to the effects of catecholamines and corticosteroids (Marco *et al.*, 1997; Marco and Lavin, 1999). ALT, AST and CK showed significantly higher values in hares without blindfold, as reported by other Authors these enzymes result high in many stressed wild animals that have been running and tends to continue rising even after the end of the action of the stressing factor (Bateson and Bradshaw, 1997; Montané *et al.*, 2003). The high significant differences between the two groups of hares could be explained by the over-exposition to the stress. In our case the physical restraint associated with the handling operations to remove the hares from the net and the restriction to take the blood are very stressful experiences for hares, particularly for the animals that are also subjected to the visual presence of the human predator. In the real practice only a sample of hares is subjected to blood drawing for sanitary monitoring, so that the effect of the stress may be less strong in most of hares, which suffer the removing operation from the net. The increase level of urea and total protein in hares without blindfold could be attributed to the effect of glycocorticoides that induce changes in the protein metabolism.

The haemocromo-cytometry of the hare showed greater concentrations in the captured hares without the blindfold than the captured hares with blindfold (Table 2). The RBC count was comparable to that observed by Marco *et al.* (2003), while the WBC and the platelets were significantly higher in our captured hares than those obtained by farmed hares used by Marco *et al.* (2003). Consequently also the plasma indexes obtained from the animals of this study were different from the hares monitored by Marco *et al.* (2003). Our data were comparable to those relieved in Poland by Nicpoń *et al.* (2007) in hares caught alive in their natural environment. The effect of the chase can explain the differences between Authors. For this reason the data of Marco *et al.* can be used as reference values only for captive hares, while our data with those of Nicpoń *et al.* (2007) better express reference values for the wild European brown hares.

Our results showed differences between groups for WBC, RBC, Hgb, and, consequently, haematocrit. The first step in stress response is the activation of the sympathetic nervous system (Montané *et al.*, 2003). The stimulation of adrenal medulla and the consequent release of catecholamines cause changes in the physiology of the animals. Increases of RBC, haemoglobin concentration and Hct are associated with splenic contraction caused by the effect of catecholamines on the spleen and on the reduction of the plasma volume (Montané *et al.*, 2003). The significant higher values observed in our study in the hare without blindfold confirms the significant reduction of the stress obtained with the use of blindfold.

The incidence of sub-clinically stressed hares and non-stressed hares, determined by the use of the discriminant function based on CK, AST and Glucose (Paci *et al.*, 2006), was 26% in the hares equipped with the blindfold and 81% in the control hares, without blindfold (χ^2_c =32.98, P<0.0001) (Figure 2). The presence of sub-clinically stressed hares even if equipped with the blindfold is probably due to the fact that stress can be avoided in the hares only after their trapping and eye blindfolding.

Table 2. Haemocromo-cytometric parameters of the hares after capture.

	Hares with blindfold N=38		Hares without blindfold N =81		
	Mean	SE	Mean	SE	Prob.>F
WBC, K/uL	7.14	0.271	8.26	0.188	0.0009**
RBC, M/uL	8.53	0.145	9.03	0.101	0.0055**
Hgb, g/dL	18.24	0.537	20.17	0.372	0.0130*
Hct, %	46.91	0.984	49.69	0.683	0.0222*
MCV, FL	54.99	0.314	55.03	0.217	0.9166
MCH, Pg	21.38	0.559	22.34	0.388	0.1610
MCHC, g/dL	38.88	1.092	40.59	0.757	0.2005
RDW, %	12.850	0.0476	12.912	0.0330	0.2931
Plt, K/uL,	457.3	9.398	466.7	6.521	0.4160
MPV, FL	8.953	0.0197	8.933	0.0137	0.3955
Pct, %	0.40610	0.00825	0.41453	0.00572	0.4028
PDW, %	9.853	0.0515	9.907	0.0357	0.3947

SE, standard error; WBC, white blood cell; RBC, red blood cell; Hgb, haemoglobin; Hct, haematocrit; MCV, mean blood cell volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RDW, red blood cell distribution width; Plt, platelets; MPV, mean platelet volume; Pct, platelet haematocrit; PDW, platelet distribution width. *P<0.05; **P<0.01.

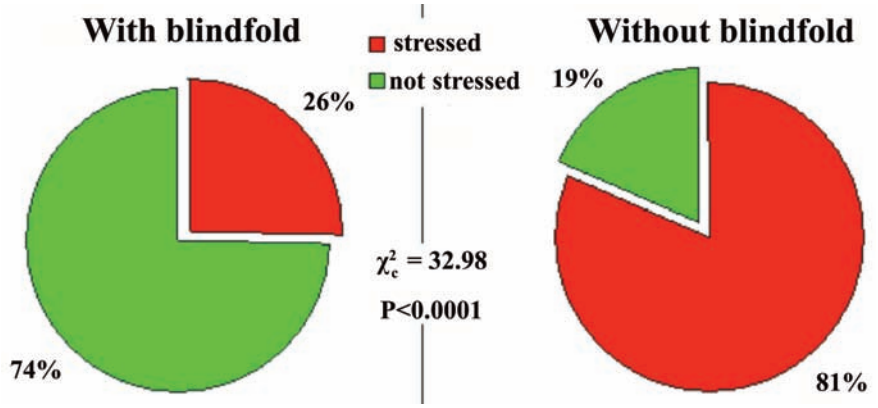

Figure 2. Different incidence of non-clinical stress in hares with or without blindfold.

The stress due to the ran of the hares chased by 3-4 (greyhounds or lurchers) coursing dogs for different times and tracks, their consequent trapping and handing before being blindfolded or not cannot be affected by the blindfolding. On the other hand, a short track, combined with quick and good handling of an experienced wildlife-officer, game wardens or hunter, can allow a significant reduction of the subclinical stress sustained by the hares captured and handled with naked eyes (Paci *et al.*, 2006).

Conclusions

Capture and handling procedures for translocation of hares should be based on protocols designed to minimize risk of myopathy to animals and risk of injury to humans. We demonstrated that the use of a blindfold applied to the hares immediately after their trapping in the net determine a significant reduction of the stress which may be heavy and directly cause the deaths of the animals and alters the normal physiology of the animals. For this reason the procedures designed for the hares translocation must always include the use of blindfolding, which should be applied as soon as possible after the trapping of the hares.

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