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ABSTRACT BOOK

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Stress evaluation in hares captured for translocation and monitored for European brown hare syndrome

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The research was carried during the capture of European hares monitored for seropositivity to EBHS-Virus. Sixty-six hares (experimental group), coming from 14 different protected areas (ZRC) of Central Italy, were subjected within few minutes after the capture to blood drawing, control of body temperature, hearth and respiratory rate. The same parameters were contemporaneously obtained in 10 <1 year old hares, reared in cages and therefore showing a reduced man-fear (control group). Blood samples (plasma) were analysed for glucose, AST (GOT), ALT (GPT), CPK and Cortisol concentrations. The presence and titration of anti-EBHSV antibodies were determined in sera of wild hares.

Seroprevalence for EBHS showed differences between areas of capture and no difference in relationship to age or sex. The parameters related to capture-stress were subjected to stepwise and discriminant analysis. CPK, AST and Glucose were selected and resulted the best parameters to discriminate the two groups i.e. all the captive hares were classified within the control group while 11 wild hares (16.6%) were not discriminated from the control group. These last wild hares were considered not stressed or with a stress level similar to that of the control/reared group and thus they were assigned to the no-stressed sample (all the captive hares + the no-discriminated wild hares). The population characteristics of the two groups (means ± Std. Error) were the following: -estimated no-stressed hares, glucose 234±9.4mg/dl, AST(GOT) 112±22.2 UI/l, CPK 1334±734UI/l; -estimated stressed hares: glucose 128±7mg/dl, AST(GOT) 163±13UI/l, CPK 4659±454UI/l.
The results obtained show that, during capture operations, most of the hares seem

to be deeply stressed and show a "muscle-damage". Since the analytes selected are cheap and quick to be determined, their determination should be encouraged and widespread adopted to better evaluate the capture operations and the relevance of operators' managing, with the aim to improve the capture techniques. The evidence of some protected areas populated only by EBHS seronegative animals and of other areas with only EBHS seropositive hares, showing variable antisera levels, often similar to those detected in vaccinated captive hares, show that the protected areas are "islands" within in the hunting territories and the final destination of the captured animals must be differentiated in relationship to their birthplace.

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STRESS EVALUATION IN HARES (*Lepus europaeus* Pallas) CAPTURED FOR TRASLOCATION AND MONITORED FOR EUROPEAN BROWN HARE SYNDROME

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INTRODUCTION

The traslocation (capture, transport and release in unknown habitats) is a stress for the animals. Intensity and duration of responses of the animals to the different operations carried out during the traslocation may differ in relationship either to the specie, to the individual within specie, or to the stressor intensity. In particular, the capture may be a heavy stress for wild animals, unpleasant experiences can directly determine "capture myopathy" and tissue damage can be showed by transient increases in the bloodstram of several enzymes and hormones. There are various physiological welfare indicators like stress hormones and other blood and physiological parameters. The selection and interpretation of welfare indicators should be based upon the knowledge of the biological functioning of animals especially the systems used by them to cope with adversity. The "stressing level" must be however determined for each parameter in each specie subjected to traslocation activities.

European Brown Hare Syndrome (EBHS) is a highly contagious, acute and fatal disease of the European Brown Hare (and not only). The disease has been firstly described in Sweden, but epidemics have been reported all over Europe. In Italy, the infection after the initial epidemic peak on late '80 has become endemic. Thus specific monitoring plan have been applied to prevent and control the diffusion of the disease.

This study was performed to determine the effect of capture stress in hares monitored for seropositivity to EBHS and traslocated from no-hunting high habitat density areas, to hunting low habitat density areas.

MATERIAL AND METHODS

Experimental group: 66 captured hares in 14 different protected areas of Central Italy. Control group: 10 captive hares. All animals were subjected to blood drawing, body temperature check and hearth and respiratory rate check. Glucose, AST(GOT), ALT(GPT), CPK and Cortisol concentrations were analysed on plasma; anti-EBHS antibodies were titrated on sera by competitive ELISA method using reference methods (OIE *Terrestrial Manual* 2004). Parameters connected to stress were analysed by the stepwise and disciminant analysis to reveal the best useful. Anti-EBHS antibodies were analysed by chi-square in relationship to the origin area.

RESULTS

Seroprevalence for EBHS showed significant differences in relationship to the capture area (P<0.05) but no difference in relationship to age or sex (Figure 1).

Figure 1 - Contingency Analysis of EBHS by area
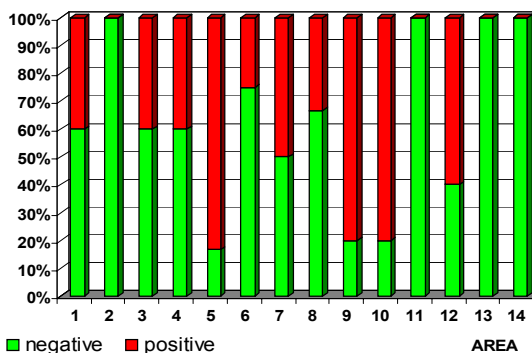
The data classified by the multivariate discriminant analysis and then submitted to the one way analysis of variance showed significant higher values of CPK and AST and the lower values of glucose in the stressed group than the no-stressed group (4659 vs. 1334 UI/l; 163 vs. 112 UI/l; 128 vs. 234 mg/dl) (Figure 2).
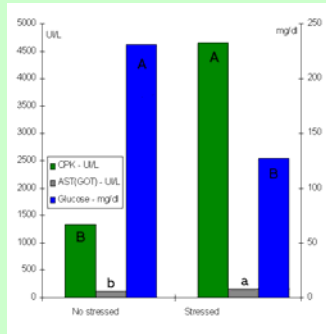

Figure 2: CPK, AST and Glucose level in stressed and no stressed hares

Glucose, CPK and AST resulted the suitable parameters to discriminate the stressed hares from the no-stressed animals (Table 1).

Table 1 - Discriminant Analysis

	F Ratio	Prob>F	Entered
T °C	0,36	0,55	
Respiratory rate	0,11	0,74	
Hearth rate	0,78	0,38	
Glucose - mg/dl	7,31	0,01	*
CPK - UI/L	6,24	0,02	*
AST (GOT) - UI/L	3,34	0,07	*
ALT (GPT) - UI/L	2,27	0,14	
Cortisol - microg/dl	0,01	0,94	

By the use of the three selected parameters all the reared animals were classified within the reared group (control: no stressed) but 11 captured hares (16,6%) were not discriminated from the reared.

These last wild hares were considered not stressed or with a stress level similar to that of the control/reared group and thus they were assigned to the no-stressed sample (all the captive hares + the no-discriminated wild hares).

By this technique were made the data of glucose, AST and CPK, reported in table 2, which can be used as reference values for no-stressed animals.

Table 2: Reference values useful to evaluate stress degree

"NO STRESSED"	Glucose mg/dl	AST (GOT) UI/L	CPK UI/L
Quantiles: maximum	352	230	4235
99.5%	352	230	4235
quartile	256	144	1619
median	228	93	1068
quartile	197	78	785
0.5%	160	60	484
minimum	160	60	484
N	21	19	21
Mean	234	112	1334
Std Dev	46,0	45,8	907,9
upper 95% Mean	255	134	1747
lower 95% Mean	213	90	921

CONCLUSIONS

The results showed that most of captured hares seem to be deeply stressed and pain related can probably also cause a "muscle damage" (showed by the observed higher values of CPK and AST) which can prejudge the hare survival rates after traslocation. Three, cheap and quickly analysable, analytes, can be used to evaluate (at least at random) the ability of the different group of operators and, consequently, can be useful to improve the capture techniques. The values obtained with the present research could be used as reference values for well captured hares (no stressed hares or hares with a stress level similar to that of the reared ones).

The presence of protected areas with only EBHS seronegative animals and the presence of protected areas with EBHS seropositive animals (even if with variable titres but similar to those of vaccinated reared hares) show that the protected areas are "islands" within in the hunting territories. Thus the final destination of the captured animals must be well evaluated and differentiated in relationship to sanitary status of both their birthplace and the area of destination.