TITLE: EFFECTS OF MYXOMA VIRUS AND RABBIT HEMORRHAGIC DISEASE VIRUS ON THE PHYSIOLOGICAL CONDITION OF WILD EUROPEAN RABBITS: IS BLOOD BIOCHEMISTRY A USEFUL MONITORING TOOL?

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ABSTRACT

Myxomatosis and rabbit hemorrhagic disease (RHD) are the major viral diseases that affect the wild European rabbit (*Oryctolagus cuniculus*). These diseases arrived in Europe within the last decades and have caused wild rabbit populations to decline dramatically. Both viruses are currently considered to be endemic in the Iberian Peninsula; periodic outbreaks that strongly impact wild populations regularly occur. Myxoma virus (MV) and rabbit hemorrhagic disease virus (RHDV) alter the physiology of infected rabbits, resulting in physical deterioration. Consequently, the persistence and viability of natural populations are affected. The main goal of our study was to determine if blood biochemistry is correlated with serostatus in wild European rabbits. We carried out seven live-trapping sessions in three wild rabbit populations over a two-year period. Blood samples were collected to measure anti-MV and anti-RHDV antibody concentrations and to measure biochemical parameters related to organ function, protein metabolism, and nutritional status. Overall, we found no significant relationships between rabbit serostatus and biochemistry. Our main result was that rabbits that were seropositive for both MV and RHDV had low gamma glutamyltransferase concentrations. Given the robustness of our analyses, the lack of significant relationships may indicate that the biochemical parameters measured are poor proxies for serostatus. Another explanation is that wild rabbits might be producing attenuated physiological responses to these viruses because the latter are now enzootic in the study area.

KEYWORDS

biochemical parameters, serostatus, myxomatosis, Oryctolagus cuniculus, physiological condition, rabbit hemorrhagic disease

INTRODUCTION

Diseases can represent major threats for wild animal populations because they can lead to decline and extinction (Viggers et al., 1993; Woodroffe, 1999; Mörner et al., 2002). In fact, acquiring a better understanding of diseases and pathogens is a crucial but challenging task in wildlife conservation efforts (Deem et al., 2001). In ecosystems, host-pathogen relationships help shape patterns of species distribution and persistence (Dobson and Hudson, 1986; Thomas et al., 2005; Collinge et al., 2006; Hudson et al., 2006). Even though most previous studies have focused on one-host, one-pathogen systems, such dynamics are actually rare in nature. Individual hosts are often co-infected by multiple pathogens, which interact in complex ways with each other (Pedersen, 2006). Therefore, studying the mechanisms underlying these interactions is of primary importance if we wish to predict how pathogens will affect host physiology and if we want to effectively control target and non-target parasite species.

Despite its relevance for wildlife conservation and management, the physiology of wild species is rarely studied because physiological parameters are difficult to quantify. Furthermore, it is challenging to combine physiological information with other data, such as antibody concentrations, at the population level. By incorporating indices of host physiological condition into population surveillance and monitoring efforts, we

will gain deeper insight into the range of host responses and pathogen effects. Such tools could reveal the status of major pathogens within wild animal populations and provide a snapshot of a given animal's physiological state; consequently, they would serve as more straightforward means of assessing population condition. In this study, we used the wild European rabbit (*Oryctolagus cuniculus*) and its two main viral diseases, myxomatosis and rabbit hemorrhagic disease (RHD), as a model system.

At present, myxomatosis and RHD are endemic diseases in the Iberian Peninsula; they cause periodic outbreaks that significantly impact natural populations (Calvete et al., 2002). Outbreak patterns suggest that these viruses are in continuous recirculation and are largely associated with the breeding season; myxomatosis outbreaks occur predominantly in summer and autumn, while RHD outbreaks occur in winter and spring. It also appears that the viruses remain in the same areas from one year to the next (Calvete et al., 2002). Factors such as breeding season length and timing, host population size, vector abundance, and environmental conditions have major effects on the duration and potential impact of the epizootics and, ultimately, on virus persistence within populations (Fouchet et al., 2008). We currently have a good grasp of the epidemiology and pathology of myxomatosis and RHD, topics that are discussed extensively in the literature (e.g., Fenner et al., 1953; Liu, 1984; Xu, 1991; Cooke, 2002; Calvete et al., 2002; Stanford et., al 2007; Abrantes et al., 2012). Myxoma virus (MV) and rabbit hemorrhagic disease virus (RHDV) dramatically alter the physiology of infected rabbits. These alterations result in the deterioration of physical health, which we will hereafter refer to as physiological condition (Kerr and Donnelly, 2013). In general, an individual's physiological condition is negatively correlated with the degree of infection burden but positively correlated with immune function (Chandra and Newberne, 1997; Gershwin et al., 1985; Møller et al., 1998). Therefore, rabbits in poor

physiological condition may also be more likely to become infected (Nelson and Demas, 1996; Tompkins and Begon, 1999; Beldomenico et al., 2008).

There is a need for straightforward, reliable methods for assessing the physiological condition of wild rabbits; past studies suggest that blood biochemistry could be helpful in this regard (Franzmann and Shwartz, 1988; Hellgrenet al., 1989; Schroeder, 1987; Hellgrenet al., 1993; Milner et al., 2003). Moreover, as compared to more conventional measures, biochemical parameters are highly sensitive, meaning they change to reflect an individual's physiological state in a matter of minutes. Consequently, the use of blood biochemistry may make it possible to identify rabbits experiencing extreme stress in general (Milner et al., 2003). In particular, Cabezas et al. (2006) revealed that biochemical parameters (e.g. urea nitrogen, total protein, creatinine and albumin) concentration changed after boosting antibodies against myxomatosis and RHD through vaccination. Therefore, we could expect that such changes may occur in a wild rabbit population when the concentration of their natural antibodies increase as a consequence of an immune response against both viruses.

In this study we monitored blood chemistry/biochemistry and MV and RHDV serostatus in wild populations of the European rabbits. Our main objectives were the following 1) to assess the usefulness of biochemical parameters as predictors of an individual's physiological condition; 2) to determine if a relationship existed between serum biochemistry and serostatus such that rabbits in poorer condition are more likely to be seropositive for MV and RHDV; and 3) to establish baseline values for biochemical parameters of rabbits with different serostatus in wild rabbit populations.

MATERIAL AND METHODS

Ethics statement

All animal experimentation was carried out in accordance with Spanish and European regulations (Law 32/2007, R.D. 1201/2005, and Council Directive2010/63/EU, R.D. 53/2013, ECC/566/2015).

Study site

The study was conducted in Hornachuelos Natural Park (100-700 m a.s.l.), which is located in a mountainous area in the southwestern Iberian Peninsula (37°49' N, 5°15' W). The climate is Mediterranean, with hot, dry summers and wet, mild winters. Three enclosures were built and used as breeding zones for rabbits. The primary objective was to increase local rabbit abundance to boost the numbers of endangered predators. The three enclosures (E1:3.8 ha; E2: 4.1 ha; E3:2.9 ha) were surrounded by 2.5-m-high chain-link fence to prevent rabbit emigration and to exclude terrestrial predators (Rouco et al., 2008). Within the enclosures, 30 artificial warrens were constructed; they followed a regular distribution pattern. Water and food pellets were supplied *ad libitum*, and grasses were sown to increase the availability of fresh food.

Sampling

From autumn 2008 to spring 2010, we conducted seven live-trapping sessions in each enclosure. Rabbits were captured using cage traps placed in the proximity of each warren, as described by Bertó-Moran et al. (2013). This methodology resulted in the capture of about 50–60% of the rabbits occupying each warren on any given night (Rouco et al., 2011).

At the trap site, captured animals were marked with individually numbered ear tags and their sex and mass were recorded. Females and males weighing more than 750 g and

850 g, respectively, were considered to be adults (Villafuerte, 1994; Alves and Moreno, 1996).

To characterize biochemical parameters and antibody concentrations, blood samples (1-2.5 ml) were collected between 900 and 1600 by venipuncture of the auricular marginal vein. The blood samples were kept at room temperature, then transported to the field laboratory where they were centrifuged on the same day as they were extracted. The serum obtained was stored at -80ºC until further analysis.

Biochemical and immunological analyses

We processed the serum samples using a COBAS INTEGRA 400 plus analyzer (Productos Roche España, Madrid, Spain).We determined the concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (BILI), lactate dehydrogenase (LDH), gamma glutamyltransferase (GGT), urea (BUN), creatinine (CREA), albumin (ALB), and total proteins (TP). These biochemical parameters are indicators of organ function, protein metabolism, and nutritional status, which means they should be good proxies for physiological condition (Harder and Kirkpatrick, 1994; Stirrat, 2003). Since they were expressed in different units, they were transformed prior to analysis to enable comparisons.

Serum concentrations of anti-MV and anti-RHDV antibodies were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits; the diagnostic techniques recommended by the World Organization for Animal Health were used (OIE, 2012), and we strictly followed the manufacturer instructions. To measure anti-MV antibodies, sera were diluted 1:40, and a relative immunity index (RI) was obtained. It was defined as a coefficient between the optical density of controls (positive and negative) and that of sampled individuals. RI values ranged from 1 to 10. All

rabbits with an RI > 2 were considered to be antibody positive (CIV TEST CUNI MIXOMATOSIS, HIPRA Laboratories, Girona, Spain). To measure anti-RHDV antibodies, the INGEZIM kit for rabbits (INGENASA Laboratories, Madrid, Spain) was used. Sera were screened using dilutions of 1:200, 1:400, 1:800, and 1:1,600. Samples with optical densities > 0.3 were considered to be antibody positive, since such antibody concentrations should be sufficient to confer protection against the disease (see Bertó-Moran et al., 2013). The test's specificity and sensitivity were 83.1% and 98.5%, respectively, and there was a 93% correspondence with the reference technique (OIE, 2012). Biochemical and immunological analyses were performed by the Physiological Ecology Laboratory of the Doñana Biological Station (Seville, Spain).

Data analysis

All statistical analyses were performed using R version 3.0.1 (R Core Development Team, 2013). Employing generalized linear mixed models (GLMM, glmer function, lme4 package) with a binomial distribution and a logit link function, we tested the relationships between the different biochemical parameters and serostatus for individuals in the three enclosures. To reduce heterogeneity, we limited our analyses to adults. Three sets of analyses were performed: 1) using rabbits seropositive for MV; 2) using rabbits seropositive for RHDV; and 3) using rabbits seropositive for both MV and RHDV. To avoid possible confounding effects, in all the analyses, we considered that individuals were seronegative only if they had neither anti-MV nor anti-RHDV antibodies. Correlations among biochemical parameters were tested, and ALT, GGT, BILI, CREA, BUN, and TP were retained as predictor variables in the subsequent

191 analyses. Serostatus was the response variable. We also included sex and rabbit density
192 as predictor variables in the models. Some individuals were sampled more than once by
193 chance. To account for the increase in type I error (rejection of the null hypothesis when
194 it is true) due to pseudoreplication (Hurlbert 1984), we included the following random
195 variable: capture session nested within individual identity nested within enclosure
196 number.

197 Prior to running the analyses, all the numeric predictor variables were scaled (except for
198 "sex") using the scale function so that their relative importance could be compared. We
199 selected the best-fit models via backward stepwise selection (anova function with
200 maximum likelihood, Crawley 2012; $p < 0.05$ as the threshold value). Each of the final
201 models contained only the significant predictors.
202

203 **RESULTS**

204 Through the course of the study, we got samples from 823 adult rabbits (306, 269, and
205 248 rabbits in E1, E2, and E3, respectively). A total of 366 samples were seropositive
206 only to MV, 124 samples were seropositive only to RHDV, 206 samples were
207 seropositive to both, MV and RHDV, and 321 samples were seronegative. Some
208 individuals were sampled more than once, and not always had the same antibody titres,
209 that is why the number of samples obtained does not math with the total number of
210 individual animals handled.
211 None of the biochemical parameters analyzed were significantly associated with MV or
212 RHDV serostatus. The only significant relationships we found were a positive
213 association between rabbit density and MV seropositivity ($p < 0.001$; Table 1) and a

214 negative association between GGT levels and seropositivity to both viruses ($p < 0.05$;
215 Table 1).

216 Each enclosure displayed different seroprevalence patterns (Figure 1). In E1, the
217 percentage of rabbits seropositive for MV, RHDV, or both remained fairly constant
218 over time. More specifically, MV seroprevalence was high for most of the trapping
219 sessions. In contrast, RHDV seroprevalence was low; it peaked at 24.4% in session 3. In
220 E2, the percentage of seronegative rabbits was generally higher than in E1 and E3, with
221 values reaching a maximum of 63.8 and 69.4% in sessions 1 and 2. MV seroprevalence
222 increased from session 3 to session 7, whereas the percentage of seronegative rabbits
223 clearly declined. Remarkably, no rabbits were seropositive for RHDV in session 7. In
224 E3, there was a higher percentage of individuals that were seropositive for both viruses,
225 as compared to E1 and E2. It was also the enclosure with the lowest percentage of
226 seronegative rabbits; this value climbed as high as 55.6% in session 4. Notably, there
227 were no seronegative rabbits in sessions 1 and 7. Rabbits seropositive for MV and for
228 RHDV were observed in every session, but their percentages were rather low. RHDV
229 seroprevalence peaked in all three enclosures in session 3.

230 In general, the ranges of values observed for the biochemical parameters remained fairly
231 consistent, although some noticeable changes in certain parameters occurred during
232 certain capture sessions (Figure 2). In E1, most of the biochemical parameters had
233 relatively constant values, but GGT and BILI fluctuated slightly. The pattern in E2 was
234 more heterogeneous. Almost all the parameters varied somewhat, except for BUN, TP,
235 ALB, and BILI. In the case of the transaminases—ALT, AST, and GGT—maximum
236 values occurred in sessions 2, 5, and 7. CREA levels were fairly constant over time but
237 hit a low in session 3, which coincided with the minimum values for the transaminases.
238 Blood biochemistry patterns were most distinct in E3. As in E2, BUN, TP, ALB, and

BILI varied little while the transaminases and CREA fluctuated dramatically. ALT and AST followed parallel patterns, both peaking in sessions 4, 6, and 7 and dropping to their minimum values in session 2. GGT presented an irregular pattern—levels were highest in session 4 and dipped down in sessions 3, 5, and 7. While CREA tended to remain constant, it dropped sharply after peaking in session 5.

Table 2 provides the means for the different biochemical parameters for the different enclosures and seropositivity classes; it also gives more detailed information related to the aforementioned patterns.

DISCUSSION

To our knowledge, this is the first study conducted in the field to address the relationship between MV and RHDV seropositivity and the physiological status of wild European rabbits using large numbers of animals and in the context of a long-term monitoring program.

In light of the results, we found limited evidence for an association between blood biochemistry and serostatus in wild European rabbit populations. The only significant relationship we observed was that rabbits seropositive for both MV and RHDV had lower GGT concentrations (Table 1). However, the lack of significant findings might be due to spurious results generated by data heterogeneity and the presence of confounding variables.

One major methodological handicap is the scarcity of data on wild rabbit populations. Most studies dealing with myxomatosis and RHD have focused on disease pathology and epidemiology in domestic rabbits. Consequently, most of the information currently available has been obtained using rabbits reared under laboratory conditions (Calvete et

al., 2002; Calvete et al., 2005; Cabezas et al., 2006; Kerr, 2012). However, physiological data for domestic rabbits is not directly comparable to that for wild rabbits since major differences exist in genetics, environmental contexts, breeding conditions, individual responsiveness, and even laboratory processes and techniques. In addition, laboratory rabbits usually develop physiological problems and specific pathologies as a result of living in captivity. These limitations aside, our results suggest that myxomatosis and RHD have declined in severity because they have become endemic in the Iberian Peninsula (Ross et al., 1986; Ross et al., 1989; Marchandeau et al., 1999; Calvete et al., 2002; Marchandeau et al., 2014). Endemic diseases have strong initial effects and cause high mortality rates in afflicted populations. However, the individuals that survive experience constant reinfections over time, ultimately leading to high immunity levels within populations. As a result, individuals become partly protected and most show mild clinical symptoms throughout the year. The pathogen can then be said to be in permanent circulation and to have become enzootic (Calvete et al., 2002; Cooke, 2002; Fouchet et al., 2008). This state of affairs is consistent with our results (Figure 1). Although the three enclosures exhibited some distinct differences, in general, there were always some individuals seropositive for MV, RHDV, or both throughout the study period. This finding suggests that the two viruses are now endemic in the study populations. It is also worth noting the fluctuating percentage of seronegative rabbits seen in E3: there were no seronegatives at the beginning or at the end of the study period. In E2, no RHDV-seropositive rabbits were found in the last capture session. Individuals with severe RHDV infections might have died, leaving no seropositives in the population; consequently, new outbreaks may result in high mortality rates. This pattern might be linked to the severity of RHD and its relatively more recent arrival, as compared to myxomatosis.

When we looked at the results for rabbit biochemistry and serostatus in tandem for the different enclosures, we observed that both were highly homogenous in E1. In E2, transaminases and CREA peaked in session 2, which was when the percentage of seronegative rabbits was the highest. The number of seronegative rabbits declined over subsequent sessions, while rabbits seropositive for MV, for RHDV, and for both became more abundant. One possible explanation is that E2 rabbits were exposed to the viruses around the time of session 2 (there were a number of outbreaks that season, as described in the literature [i.e., Calvete et al., 2002]), which is suggested by the session 2 peak in transaminases. In sessions 3 and 4, the number of seropositive rabbits increased and both the transaminases and CREA dropped to their minimum values. This result lends support to the idea that rabbits that have been exposed to the viruses, and that consequently develop immunity, are likely to return to basal biochemical parameter values.

In E3, transaminases peaked in session 4, which is also when the number of seronegative rabbits was highest. In the subsequent capture sessions (sessions 5 and 6), the percentage of seronegative individuals declined sharply while the number of individuals seropositive for MV, RHDV, or both climbed. This pattern probably resulted from a high incidence of the diseases in session 4 and earlier. The population's exposure to the viruses can be seen in the increase in transaminases in session 4, which is when they reached maximum levels. In sessions 5, 6, and 7, after rabbits had become seropositive, the transaminases were close to their minimum levels, suggesting that immune (seropositive) rabbits tended to return to basal parameter levels.

As in E2, in E3 there were large numbers of seronegative rabbits in sessions 2 and 3, just before transaminases peaked in session 4, which likely signaled the beginning of an endemic disease cycle.

313 Of the biochemical parameters studied, the transaminases (ALT, AST, and GGT) were
314 clearly the most variable for all three enclosures. This pattern may reflect the impaired
315 hepatic function seen in rabbits infected with MV and/or RHDV.

316 In addition to the shortcomings mentioned above, the lack of significant findings puts
317 into question the utility of biochemical parameters in assessing the physiological
318 condition of European rabbits. As is clear from the literature, serum biochemistry might
319 be influenced by a variety of factors, including rabbit handling and sampling
320 procedures, fieldwork conditions, and animal nutritional and health status at the time of
321 sampling (Calvete et al., 2005; Cabezas et al., 2006). Furthermore, there is individual-
322 level variation in immune and physiological responses as a result of trade-offs between
323 environmental conditions and life-history traits (e.g., developmental, physiological,
324 genetic, and immunological traits) (Ardia et al., 2011). Therefore, alternative indicators
325 such as concentrations of specific immunoglobulins (e.g., IgM or IgG) or cellular
326 oxidative stress markers could provide more complete and precise information.

327 As discussed above, confounding variables that were not accounted for in our analyses
328 could be skewing our results. Such variables could include the following: (1) rabbit age;
329 (2) outbreak timing; (3) the ELISA seropositivity thresholds; (4) the response speed of
330 biochemical parameters; and (5) the lack of reference values for wild rabbits.

331 One major factor could be rabbit age. In this study, we estimated age based on mass.
332 Although this approach can separate adult rabbits from non-adult rabbits, it cannot
333 reveal a rabbit's precise age. Knowing a rabbit's age could be important because as
334 rabbits get older, their probability of being infected by a wide variety of potentially
335 serious pathogens like MV or RHDV increases, as do antibody levels (Marchandeau et
336 al., 1995; Parkes et al., 2002; Parkes et al., 2008). Furthermore, a rabbit's innate

responsiveness changes over its lifetime, which means that individuals of different ages will have different biochemical profiles and immunological experience.

Outbreak timing is also important but difficult to characterize. Myxomatosis and RHD outbreaks show some seasonal and geographic variation (Mutze et al., 2008; Mutze et al., 2010; Abrantes et al., 2012). More specifically, the occurrence of epizootics might vary across years and even among populations (i.e., enclosures) as a result of delayed breeding and variable climatic conditions, which can affect the abundance and activity of the pathogens' vectors. Determining the moment of infection is nearly impossible, so outbreak timing is only approximate.

As mentioned above, wild rabbits are naturally exposed to a wide variety of pathogens, whereas laboratory rabbits are artificially infected with a smaller selection of them. The ELISA techniques that we used to determine MV and RHDV seropositivity were developed using European rabbits kept under laboratory conditions. It may be that applying such seropositivity thresholds to wild rabbits could yield false positives and cross-reactions since laboratory rabbits are exposed to fewer pathogen species and thus have lower threshold antibody concentrations than wild rabbits (Kerr, 1997).
Finally, the response speed of biochemical parameters must be accounted for. Serum biochemistry changes are relatively transient, as demonstrated by several studies in which rabbits were artificially infected with pathogens (Ferreira et al., 2004). Rabbits show an initial physiological response to infection, but if they do not die, any changed biochemical parameters revert to their basal values. Nevertheless, such shifts are likely to go undetected in the wild.

In conclusion, it will be important to carry out further research that explores straightforward, reliable indices that can be used to assess the physiological condition of

individuals in target wildlife populations. Selecting the right methods and biochemical parameters is essential if we wish to more rapidly detect and control diseases in wild species, which would help improve management and conservation programs.

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Table 1. Results for the generalized mixed models for each dataset (i.e., MV: rabbits seropositive for myxoma virus; RHDV: rabbits seropositive for rabbit hemorrhagic disease virus; MV & RHDV: rabbits seropositive for both viruses). Coefficient estimates (β), estimated standard errors (SE), and p-values (p) are listed.

	MV			**RHDV**			**MV & RHDV**		
	B	SE	p	β	SE	p	β	SE	p
ALT	0.2042	0.1241	0.09433	0.1940	0.1646	0.2486	0.2391	0.1362	0.08241
GGT	0.01504	0.11533	0.8961	-0.06207	0.17053	0.7131	-0.3031	0.1599	0.0491*
BILI	-0.07875	0.10509	0.453	-0.2079	0.1636	0.192	-0.1320	0.1427	0.3639
CREA	-0.04941	0.10756	0.6478	-0.03273	0.17178	0.8467	-0.1195	0.1651	0.4781
BUN	0.04409	0.10948	0.686	-0.01682	0.14124	0.9006	0.07518	0.11388	0.5182
ALB	-0.04380	0.11618	0.7065	0.02039	0.16918	0.8993	0.08296	0.14723	0.5847
density	0.3728	0.1112	0.000804*	-0.03413	0.17652	0.8461	0.01127	0.14411	0.9383
Sex	0.2173	0.2155	0.3112	0.10556	0.29962	0.7248	0.3539	0.2554	0.1703

Table 2. Blood biochemistry of wild European rabbits in the three study enclosures; rabbits are grouped by serostatus (seronegative, seropositive for myxoma virus [Myxo+], seropositive for rabbit hemorrhagic disease virus [Rhd+], and seropositive for both viruses [Myxo+/Rhd+]). Values correspond to the mean ± SE.

	E1				**E2**				**E3**			
Parameter(units)	***seronegative***	***Myxo+***	***Rhd+***	***Myxo+/Rhd+***	***seronegative***	***Myxo+***	***Rhd+***	***Myxo+/Rhd+***	***seronegative***	***Myxo+***	***hd+***	***Myxo+/Rhd+***
ALT (U/L)	9±0.7	8.5±0.9	9.6±0.5	10.1±0.9	12.6±0.8	14.7±1.8	13.8±1.0	15.1±2.0	19.6±2.1	20±2.0	21±1.4	20.3±1.2
AST (U/L)	32.2±3.2	31.4±3.2	31.9±1.9	38.1±5.0	44.8±3.0	46.4±6.3	48.3±4.1	39.8±3.9	66±9.1	56.8±6.1	60.8±4.0	65.3±5.1
GGT(U/L)	6.9±0.6	7±0.7	7.4±0.4	7.1±0.6	9.2±0.6	8±1.1	8.4±0.5	8±0.8	9.2±0.9	9.3±0.9	10.3±1.3	7.4±0.4
BILI (µmol/L)	1.4±0.1	1.2±0.1	1.4±0.1	1.3±0.1	1.7±0.1	1.6±0.1	1.7±0.1	1.5±0.2	1.7±0.2	1.7±0.1	1.8±0.1	1.7±0.1
CREA (mg/dl)	0.8±0.04	0.8±0.07	0.8±0.02	0.8±0.05	0.9±0.04	0.8±0.07	0.8±0.05	0.9±0.1	1±0.2	0.9±0.1	1±0.1	0.9±0.05
BUN(mg/dl)	73.7±4.8	76.2±7.9	74±2.2	71.9±3.4	70.4±2.6	66±4.9	77.7±2.3	73.2±5.7	76.5±5.1	77.7±3.3	72.4±3.3	79.8±3.4
LDH(U/L)	643.1±43.5	647.7±34	697.8±44.0	697.4±64.6	670.5±37.9	582.9±52.4	703±36.9	740±91.8	890±98.0	743.2±95.7	918.9±73.2	862.4±63.5
ALB(g/dl)	4.8±0.1	5±0.2	4.7±0.1	4.7±0.1	4.9±0.1	4.5±0.2	4.9±0.1	5±0.1	4.5±0.2	4.7±0.1	4.7±0.1	4.6±0.1
TP (g/dl)	5.7±0.2	6.1±0.4	5.7±0.1	6±0.4	5.8±0.1	5.4±0.2	5.8±0.1	5.7±0.3	5.4±0.2	5.7±0.1	5.8±0.1	5.8±0.1

525

FIGURES CAPTIONS

Figure 1. Variation in MV and RHDVseroprevalence in rabbit populations (E1, E2, and E3) over the two-year study period

Figure 2. Transformed values (mean ± SE) of biochemical parameters for each capture session in the three study enclosures (E1, E2, and E3).Alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (BILI), lactate dehydrogenase (LDH), gamma glutamyltransferase (GGT), urea (BUN), creatinine (CREA), albumin (ALB), and total proteins (TP)

532 FIGURE 1

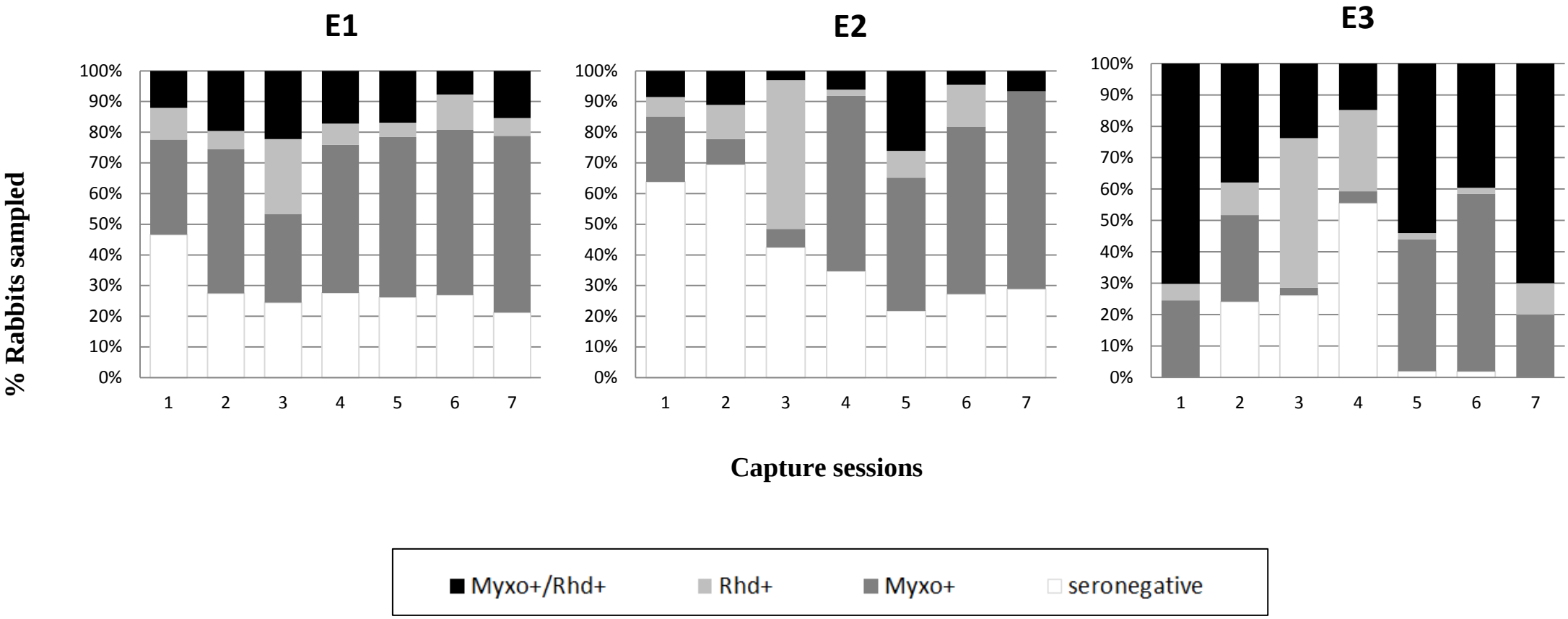
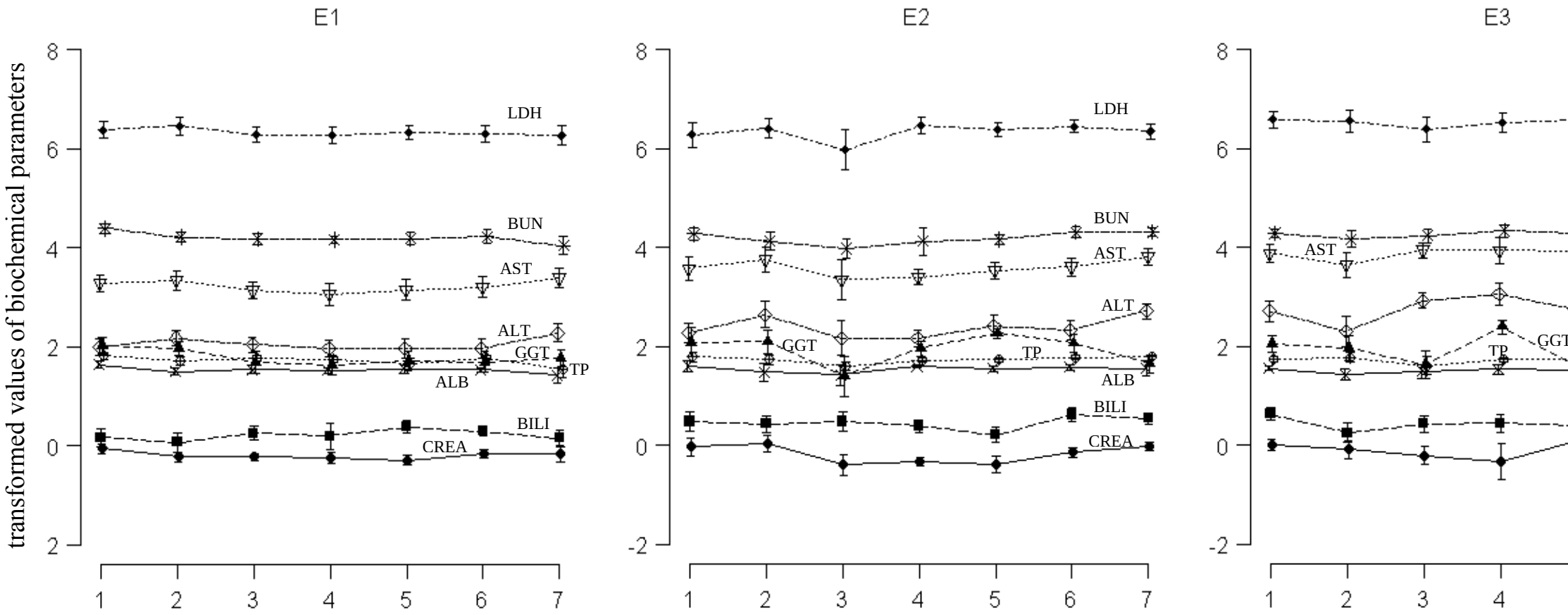

E1
E2
E3
% Rabbits sampled
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1
2
3
4
5
6
7
Capture sessions
Myxo+/Rhd+
Rhd+
Myxo+
seronegative

FIGURE 2


Capture sessions
transformed values of biochemical parameters
E1
E2
E3
LDH
BUN
AST
ALT
GGT
TP
ALB
BILI
CREA