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Differential acute phase protein response induced in mice experimentally infected with *Brucella melitensis* 16M or with the vaccine strain Rev1.

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Brucellosis affects at different species of animals and is zoonotic for humans. Vaccines are based on live attenuated bacteria and animals develop the same antibodies being impossible to distinguish serologically vaccinated animals from infected[1]. Acute phase proteins (APPs), used as biomarkers[2], could be useful to solve this problem. THE AIM OF THIS WORK is to characterize the APPs response in mice infected with *B. melitensis* 16M or vaccinated with Rev1 to detect biomarkers differentiating between two conditions.

MATERIALS and METHODS

Kinetics of infection. Female balb/c mice (4 per lot) were intraperitoneally (ip) injected with 10^4 colony forming units (CFU) of *B. melitensis* 16M or with the vaccine strain Rev1. Rev1 was also subcutaneously (sc) injected at 10^5 CFU since it is the standard route and dose for vaccine administration. Control mice were ip injected with PBS. Weight and CFU from liver and spleen were determined at 7, 14, 21, 28 and 56 days. ***In vitro* studies**. Serum concentration of serum amyloid A (SAA), α-1 glycoprotein acid (AGP), haptoglobin (HPT) and hemopexin (HPX) were measured by ELISA. Liver and spleen mRNA relative units (RU) of this APPs were determined by qPCR. All **data** are represented as Mean±SEM and were analysed using independent samples t-Test with SPSS 16.0. *p<0.05, **p<0.01, ***p<0.001.

RESULTS and DISCUSSION

Differences were found in APPs serum concentration among mice injected with *B. melitensis* 16M, Rev1 ip and Rev1 sc. These differences were also reflected in liver mRNA levels of these proteins.

Peaking on day 14, AGP, HPT and HPX serum concentration were greater in mice injected with Rev1 ip, intermediate with *B. melitensis* 16M and similar to control with Rev1 sc. The peak of SAA was also reached on day 14 for mice inoculated with Rev1 ip, but did it earlier (day 7) for mice inoculated with *B. melitensis* 16M. These SAA values were higher than those for Rev1 on day 3 (sc) and 7 (ip or sc) (Figure 1).

APPs serum concentration values were in agreement with spleen and liver inflammation (weight) caused by any of these inoculations. Maximum CFU values were reached before of those of inflammation, being 1.5 fold lower for Rev1 sc (Table 1).

APPs gene expression was upregulated in liver and downregulated in spleen, except for SAA in the latter. Time-course of liver APPs gene expression was peaked on day 14 (or day 7 for *B. melitensis* 16M). Mice inoculated with Rev1 sc showed a high level of APPs mRNA that did not correspond to the protein concentration in serum. This effect was more evident for HPX (Figure 2). Thus, Rev1 sc inoculation could cause protein malformations or inhibition of the APPs secretion mechanism by liver. Spleen did not contribute to mRNA synthesis of APPs except for SAA. AGP, HPT and HPX were downregulated on day 7 post-inoculation. A recovery of their expression was observed at 14-21 days, except for those injected with *B. melitensis* 16M (Figure 3).

Table 1. Number of *Brucella* (CFU) and liver and spleen weight (g). Mean±SEM; n=4.

Spleen	Time post-inoculation	3 days	7 days	14 days	21 days	28 days	56 days
log CFU	***B. melitensis* 16M**	5.19±0.07	6.27±0.07	5.00±0.11	4.73±0.12	4.66±0.39	3.55±0.52
	Rev1 ip	4.71±0.12	6.53±0.06	5.65±0.16	4.87±0.18	3.42±0.36	2.93±0.61
	Rev1 sc	1.20±0.71	3.45±0.58	4.40±0.42	3.67±0.72	2.69±0.69	0.00±0.00
g	***B. melitensis* 16M**	0.14±0.01	0.23±0.02	0.31±0.04	0.25±0.02	0.22±0.02	0.15±0.01
	Rev1 ip	0.11±0.01	0.12±0.01	0.45±0.04	0.33±0.06	0.21±0.01	0.20±0.01
	Rev1 sc	0.11±0.11	0.14±0.14	0.20±0.03	0.17±0.02	0.17±0.02	0.13±0.01

Liver	Time post-inoculation	3 days	7 days	14 days	21 days	28 days	56 days
log CFU	***B. melitensis* 16M**	4.72±0.06	4.79±0.07	3.15±0.16	2.54±0.29	2.25±0.79	0.99±0.57
	Rev1 ip	4.78±0.07	4.81±0.17	3.02±0.26	2.34±0.78	1.43±0.52	0.36±0.36
	Rev1 sc	0.48±0.48	2.64±0.29	2.90±0.14	1.72±0.62	1.48±0.60	0.56±0.56
g	***B. melitensis* 16M**	1.10±0.09	1.41±0.05	1.20±0.11	1.38±0.06	1.36±0.05	1.34±0.14
	Rev1 ip	1.09±0.08	1.06±0.03	1.52±0.06	1.40±0.09	1.24±0.06	1.33±0.07
	Rev1 sc	1.14±0.07	1.11±0.05	1.20±0.07	0.94±0.11	1.00±0.06	1.22±0.06

Figure 1. Time-course of serum concentration of **SAA**, **AGP**, **HPT** and **HPX**. Mean±SEM; n=4. */**/*** *vs* Rev1 ip; */**/*** *vs* Rev1 sc.

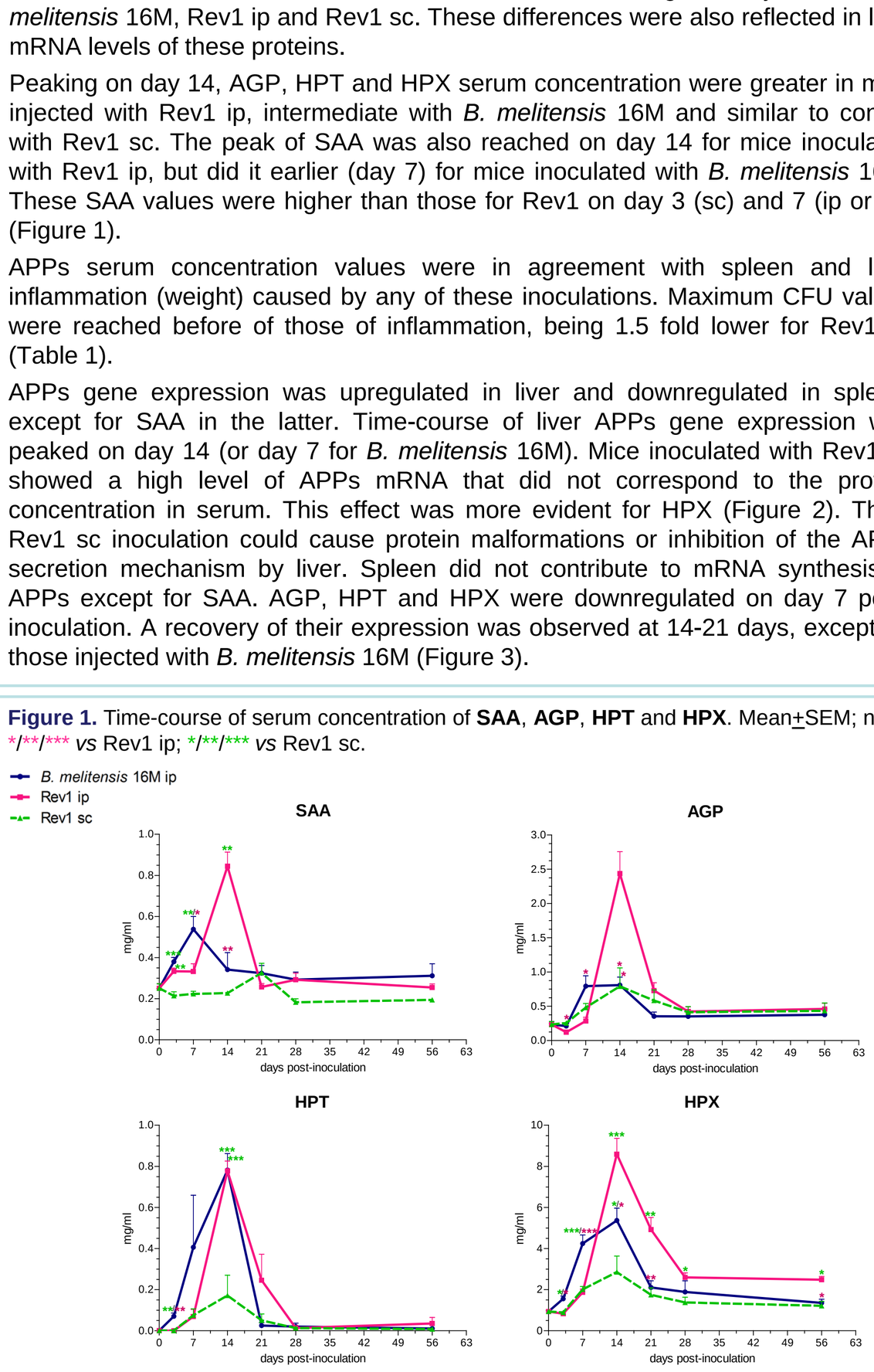


Figure 2. Time-course of liver mRNA level of **SAA**, **AGP**, **HPT** and **HPX**. Mean±SEM; n=2.

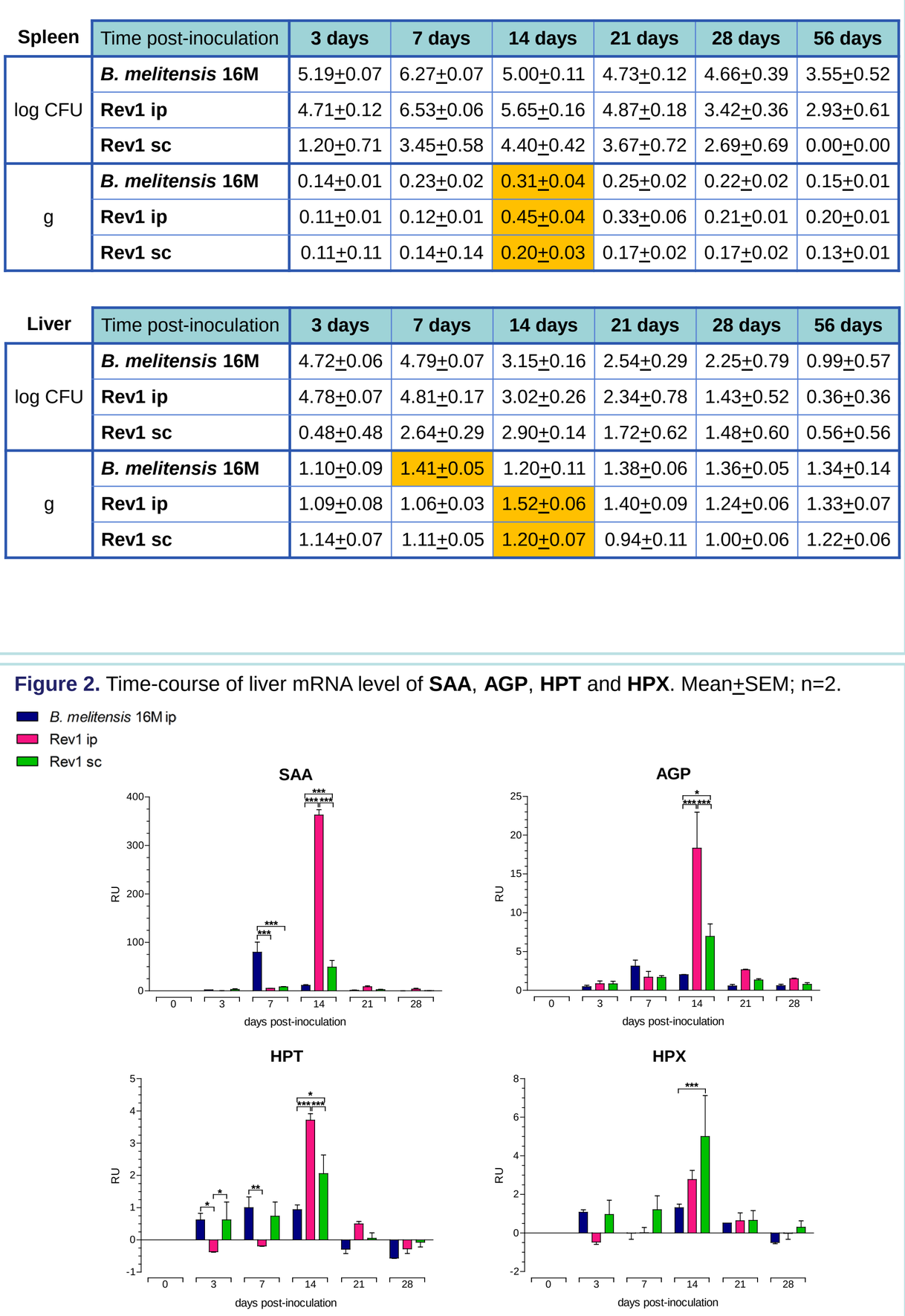
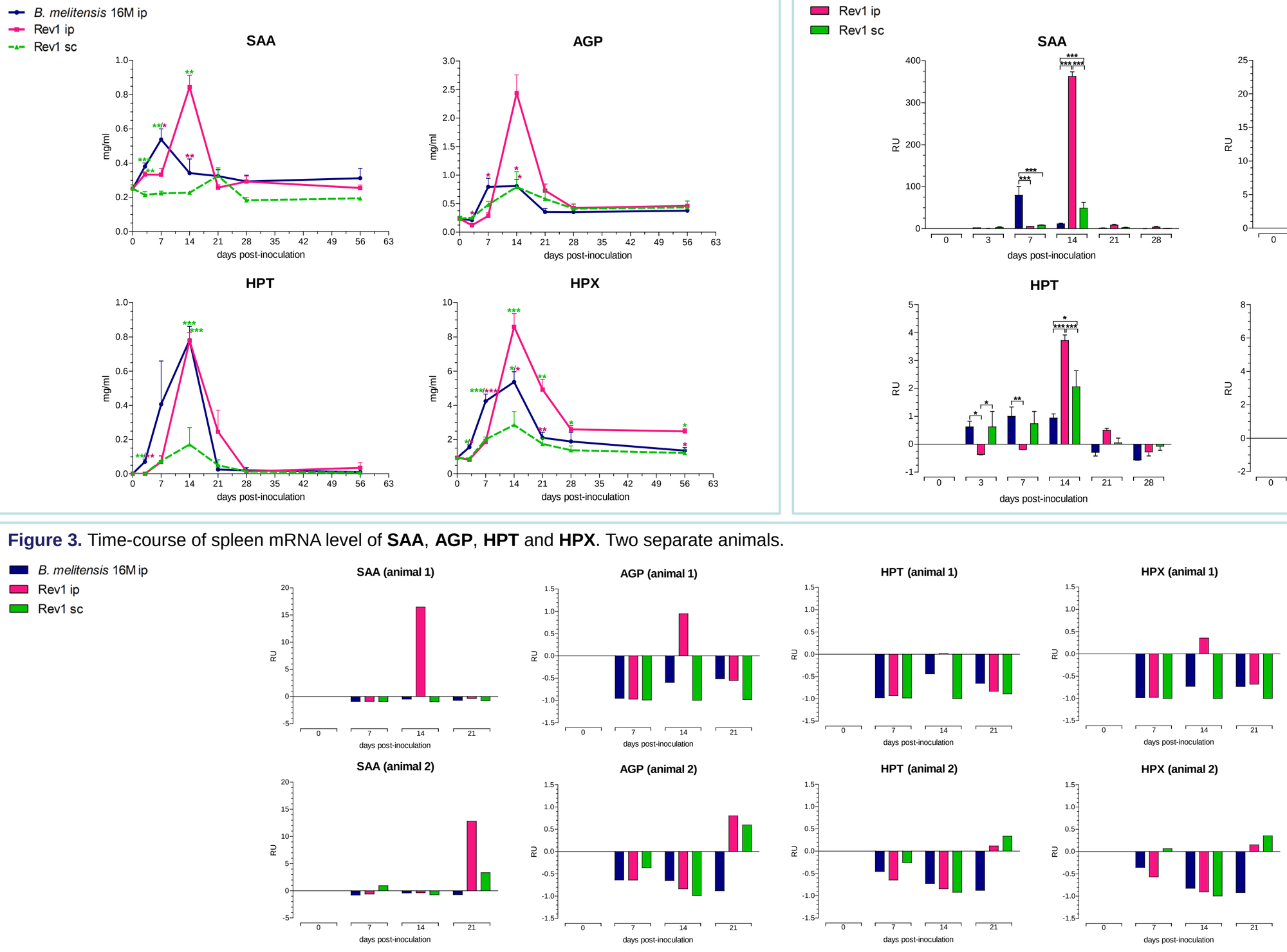


Figure 3. Time-course of spleen mRNA level of **SAA**, **AGP**, **HPT** and **HPX**. Two separate animals.



CONCLUSION: APPs show great promise as biomarkers to differentiate between *Brucella melitensis* infected and vaccinated animals. Specifically, HPT and HPX during medium term and SAA during the early stage of *Brucella*-induced inflammation.

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