Safety and Effectiveness of Paramunization in Import Reptiles

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1 INTRODUCTION

Reptiles are kept in captivity and exploited for commercial and non-commercial reasons all over the world. In most cases they are exposed to immunesuppressive distress and microbial pressure through catching, handling, crowding, grouping, shipping and suboptimal keeping conditions (STEINMETZ et al., 1998). Beyond that anthropozoonotic circumstances reptiles themselves are sources for microbial and viral danger to people in the sense of zooanthroponosis (BLAHAK, 2000).

Veterinary attempts with classic specific vaccines to immunize reptiles have failed, even ended disastrous so far. The success of the above-mentioned utilization processes may be optimized with paramunization to the benefit of reptile and human. Paramunity inducers (PIND) from attenuated animal pox virus directly activate and regulate the paraspecific (=innate) immune system. This fits excellently to a reptile immune system heavily paraspecific = reptimunsystem. Due to ectothermy and phylogenetic weakness of specific immune system reptile defense much more relies on the ten times older paraspecific immune system than in endotherm vertebrates (GUILLETTE et al., 1995; WARR et al., 1995; ZAPATA und AMEMIYA, 2000). Recently immunologists focus intensively to that old conserved basis of "immunity" to answer complex immune questions. Paramunity inducers are potentially the clue to help defend infectious diseases of reptiles and could show a future way in combining paramunity inducers and specfic antigen formulations for reptile immunization. Considering that background success with paramunity inducers and reptiles was empiric but systematic prove of compatibility and effectiveness could be verified recently by comparing paramunized and unparamunized import reptile groups.

2 MATERIAL AND METHODS

2.1 Paramunity inducer

Animal pox virus are good paramunity inducers. In passaging they very early loose the specific immunizing epitopes wheras the phylogentic older epitopes are conserved and hence the paraspecific cytokine regulating function is retained (MAYR und MAYR, 1999; MAYR, 2001). We used the paramunity inducer PIND-AVI, 444th FHE passage of the avipox virus stem HP1. As "start material" for our preparations we utilized virus cultured on chicken embryo fibroblasts (FHE), finally inactivated with ß-propiolakton. Quality control showed PIND-AVI contained > 320 units/ml (VSV-Challenge). A labelled paramunity inducer ZYLEXIS (formerly BAYPAMUNE), containing PIND-ORF cultured on bovine tissue is available in Germany and efforts are obtained for registration in other countries. Table 1 enlights the difference between paramunization and immunization

Table1: Difference between paramunization and immunization

type	active agents	protective agents	effect	time	postvaccinal damage	definition
conventional specific vaccine immunizing	specific immunizing epitopes	antibodies immunecells	specific protecting vaccination specific immunization	begins within 5-8d lasts months-years	vaccination disease allergies, autoimmune disease, immune complex disease	active vaccination
paramunity inducer regulating	non immunizing regulating proteins	macrophages lymphoreticular cells, cytokines	paramunity, unspecific protection	begins instantly lasts 10 - 12 d	**none**	**paramunization**

2.2 Import enterprise

The enterprise was a wholesale reptile import-export enterprise in southern germany. The reptile turnover was 13 days and loss and reclamations were lower than typical , in 2003 1.7%. For reptiles the postimport mortality in wholesale operations is estimated with 3 to 4%, which is about the same figure as transport mortality (STEINMETZ et al., 1998). Selection criteria for

study groups was availibility, manageability and in view of **mortality,** lability of groups. As there were not enough labile groups available, also stabile groups were added to the pool and for validation the indicator **reptile fitness score** (**RFS**) introduced. With the positive followup of a **skin ulcer** disease in an *Acanthosaura capra* group there was a third indicator. After prestudies to confirm compatibility, groups were randomized in verum and control groups and after one or two weeks the indicators surveyed and statistically validated.

2.3 Indicator mortality

The vivariums were marked with encoded stickers and death cases were documented with tally sheets. With difference calculation the result was verified after day 7 or day 14.

2.4 indicator RFS

Reptile-Fitness-Score (RFS) expresses sales quality minus adjectives not health relevant like beauty, old scars, regenerated tissue, shedding, size and sex. Groups were introduced to the manager anonymously. After individual inspection every animal was scored between 1 and 5.

2.5 STATISTICS

The level of significance was calculated with the statistic software SAS-System® Version 8.2. A difference was accepted if $p < 0,05$.

3 REPTILES

The study comprised 493 reptiles with 12 species from 5 continents. Studied import reptile groups wer wild caught, ranched or farmed, of different age, body mass, condition and sex and exposed to more or less shipping distress. Mass spanned from 2 to 300 g. Table 2 lists the reptile species in this study.

Tabelle 2: reptile species in the paramunity study

scientific name	common name
Agama agama	Red-headed Rock Agama
Agama atricollis	Blue-headed Tree Agama
Acanthosaura capra	Horned Mountain Dragon
Basiliscus vittatus	Brown Basilisk
Sceloporus variabilis	Rosebelly Lizard
Callisaurus draconoides	Zebra-tailed Lizard
Sauromalus obesus	Chuckwalla
Bronchocela jubata	Maned forest lizard
Graptemys kohnii	Mississippi Map Turtle
Iguana iguana	Green Iguana
Crotaphytus collaris	Collared Lizard
Physignathus cocincinus	Chinese Water Dragon

Reptiles were restrained in semidarkness (bulbs and floods detached) to reduce escape and defense behaviour and placed in plastic containers. The inducer was transported lyophilized at 4 to 6 ° C and diluted with AMPUWA® (pyrogen free injection water) immediate before application. 0,1 ml of inducer or physiologic salt solution as placebo was applicated with 1 ml tuberculin syringe. As the depth of injection was difficult to control an appopriately cut plastic teat canula limited injection depth to 2 mm. Injection was placed in direction of scales orientation to minimize reflux of injected volume.

4 RESULTS

All injected reptiles form group S1 to S14 at every control day were free of local or systemic side effects. Inducer groups never performed worse neither quantitatively nor significantly to control groups. We didnt observe a difference in compatibility in labile or stabile groups.

Looking at that groups we expected high mortality there was always a quantitative and as a rule a significant advantage to the verum groups. Table 3 describes the findings.

Tabelle 3: comparison of significant and quantitative advantage of PIND-AVI-grpups S1 to S14

group	#groups	L/S		Msig +	Mq+	Msig -	Hgsig +	Hgsig -	RFSsig +	RFSq +	RFSsig -
Inducer Double Injection	4	L	3	S1 S6	S2	S2	S2		S2		
		S	1		S3	S3					S3
Inducer Single Injection	8	L	5	S11 S12 (S13) (S14)	S10	S10	S11		S13	S14	S14
		S	3		S4 S5 S8	S4 S5 S8			S5	S4 S8	S4 S8

caption to table 3: #groups: number of studied group pairs; character of group L/S: L = labil, S = stabil; Msig + / Msig - : mortality significant/ no significant difference, Mq+: mortality quantitative advantage; HGsig+/ HGsig- : skin ulcers significant/ no significant difference; RFSsig+/RFSsig- : RFS significant/ not significant differnce; in parenthesis: missed significance marginal. S12 = allocated to significant groups as verum group day0 was obviously ill at start compared to control group and day7 group difference was no more significant. RFSq+: quantitative advantage

Subsuming the results there was following picture: Total mortality (unstable groups), day7 RFS and percentage of day7 skin ulcers (*Acanthosaura capra*) for PIND-AVI and control groups added up to 9% and 30%, 3.58 and 2.87, 5% and 52%, respectively.

5 DISCUSSION

Compatibility could be stated in all PIND-Avi injected reptile groups. During study we could not observe any adverse local or systemic reactions from injecting 0.1 ml. Also 0.5 ml application to massier *Sauromalus obesus* or intraabdominal application to *Graptemys-kohnii*-hatchlings with 0.05 ml was tolerated without negative side effects.

Effectiveness to stressed import reptiles was evident looking at the datas. Mortality was reduced, fitness improved and a skin ulcer disease symptoms relieved within short time.

Perspective of animal pox virus derived paramunity inducers in reptile medicine to fight infections and metabolic diseases (in the context of the reptilian immuno-neuro-endocrine network) is promising. Substantial veterinary importance of microbial infections we find today in tortoises and iguanas (herpes virus), sea turtles (papilloma virus, herpes virus) and crocodiles (mycoplasma) in private collections, conservation and farm projects implicating both ecological and commercial loss. Classic vaccination attempts failed so far. In future we could use paramunity inducers alone or in combination with specific formulations to treat or protect reptiles from infectious diseases. Further indications are listed in table 4.

Tabelle 4: Supposable indication fields for paramunty inducers in reptiles

prophylactic indications for reptiles	**therapeutic indications for reptiles**
rapid activation of paraspecific innate immune system in hatchlings subadults adults and geriatic reptile patients.	infectious diseases, infectious factoral diseases, parasitosis, intoxication
before expectable distress in general	immune suppression and trauma
after catching, before and after transport, sale, regrouping exhibition	chronic recurrent diseases
before and after acute infection danger, unfavorable circumstances, after technical defects.	supporting tumor therapy
before and after hibernation, aestivation, mating season, egg deposing, food change, shedding,	reconvalescence
in combination with vaccines	with insecticide, desinfection antimicrobial therapy
before and during unfavorable feeding conditions	metabolic diseases of various etiology
to prolonge life span	chronic skin diseases
before and after iatrogen and surgical intervention	technopathies and wounds; to minimize healing complications

Further questions related to the **Reptimunsystem** should be investigated:

- does a reptimunsystem heavily paraspecific consume more or less energy ?
- does the paraspecific immune system of reptiles imply higher virtuosity than in birds or mammals?

- and does it effectively compensate weaknesses of specific immune system?
- are immune suppressions due to ectothermy all corticoid related?
- how is the immune situation during summer aestivation?
- what impact does light quality and dynamics have to a reptilian immune system?
- what aspects of the immune system make some species (turtles) robust and others (agama) fragile?

6 SUMMARY

Safety and Effectiveness of a Paramunization in Import Reptiles.

Results confirmed compatibility and effectiveness of the paramunity inducer PIND-AVI in imported reptiles and hence in ectothermic vertebrates for the first time. This field study comprised 493 reptiles with 12 species in a German import-export operation. Total mortality (unstable groups), day7 RFS and percentage of day7 skin ulcers (*Acanthosaura capra*) for PIND-AVI and control groups added up to 9% and 30%, 3.58 and 2.87, 5% and 52%, respectively. As the development of immunoregulative drugs and specific vaccines, especially for those "minor species", is out of sight and the characteristic ectothermal dynamics of the reptile immune system (reptimunsytem) makes a conventional veterinary approach difficult, there are many future perspectives with PIND-AVI to positively influence the immuno-neuro-endocrine network of reptiles.

7 LITERATURE

1	BLAHAK S. (2000)
	Infektionskrankheiten der Reptilien unter besonderer Berücksichtigung der Zoonosen - Ein Überblick für die Praxis
	Praktischer Tierarzt 81: 2, 113-26
2	BRAMES H. (2005)

	Wirksamkeit und Verträglichkeit des Paramunitätsinducers PIND-AVI bei Importreptilien
	Diss. med. vet., München
3	GUILLETTE L. J., CREE A., ROONEY A. A. (1995)
	Biology of stress: interactions with reproduction, immunology and intermediary metabolism
	In: Health and Welfare of Captive Reptiles (Hrsg: WARWICK C., FRYE F. L., MURPHY J. B.), Chapman & Hall, London
4	MAYR A., MAYR B. (1999)
	A new concept in prophylaxis and therapy: paramunization by poxvirus inducers
	Pesq. Vet. Bras. vol.19 n.3-4 Rio de Janeiro July/Dec. 1999 URL: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X1999000300001&lng=en&nrm=iso&tlng=en
5	MAYR A. (2001)
	Entwicklung einer nicht immunisierenden, paraspezifischen Vaccine aus attenuierten Pockenviren: Eine neue Art von Vaccinen
	Berl Münch Tierarztl Wochenschr. 2001
6	STEINMETZ M., PÜTSCH M., BISSCHOPINCK T. (1998)
	Untersuchung zur Transportmortalität beim Import von Vögeln and Reptilien nach Deutschland
	Bundesamt für Naturschutz (Hrsg.)
7	WARR G. W., MAGOR K. E., HIGGINS D. A. (1995)
	IgY: clues to the origins of modern antibodies
	Immunol Today. 1995 Aug, 16(8), 392-8
8	ZAPATA A. G., AMEMIYA C.T. (2000)
	Phylogeny of lower vertebrates and their immunological structures
	In: Origin and Evolution of the Vertebrate Immune System , Chapter 8, (Hrsg.: Du Pasquier L.), Springer-Verlag, Heidelberg, 67-110

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