

Article

Changes in the Stress Tolerance of Dwarf Rabbits in Animal-Assisted Interventions

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Abstract: Background: Rabbits are common companion animals in many European countries. Rabbit-assisted interventions in primary schools are suitable for decreasing the anxiety levels of children, which may increase the education efficiency of teachers. Methods: In our present study, the behavior of dwarf rabbits was tested in a children's summer camp organized by the Family and Child Protection Service in Várpalota, Hungary, 2021. In two occasions, altogether 20 rabbits were evaluated, while 120 children were involved in the examinations. All the rabbits were the progeny of the same buck and three different does. Four of them had been previously socialized to humans during the sensitive period after birth. Results: The familiarity level of the rabbits with their owner was significantly lower after the interventions compared to the previously measured scores ($p < 0.05$). In the interventions, children also made behavior surveys with the rabbits in which they contacted the animals, touched them and held them. After three successive interventions, rabbits became significantly less tolerant compared to the first intervention ($p < 0.05$). In both cases, the rabbits who had been socialized obtained higher scores on the behavior tests compared to the non-handled conspecifics. However, by the third session, socialized rabbits showed a considerable decrease in cooperation. Conclusions: The stress tolerance of rabbits receiving handling exceeds that of those that were not handled previously. The familiarity level of the imprinted rabbits was higher in both cases: with the owner and children. When using rabbits in AAI, a maximum of two 20 min sessions is recommended for handled rabbits.

Keywords: therapy rabbits; stress; animal-assisted intervention; handling



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1. Introduction

Several studies show that companion animals improve human owners' health and well-being [1]. People who are in daily contact with animals tend to have lower stress levels [2] and also find it easier to establish human relationships [3]. The damaging effects of stress are general regardless of position, social class and age [2]. Education-related challenges for children are continuously increasing. Wherever they encounter an emotional crisis, they may turn to animals because they appreciate their companionship, understanding and comforting influence [4]. As per the theory of biophilia, the explanation for the impact of animals on humans is an inherited feature [5]. The question of which animal could be considered suitable for therapy is subjective. Preliminary knowledge of the animal's behaviour, the safety of the person involved in the therapy and the well-being of the animals are all very important [6].

Animal therapy organisations use 94% dogs or cats during their interventions, but besides these species, others also appear [7]. In therapy programmes, rabbits and other small mammals (chinchillas, guinea pigs, mice) and birds are also being used [6,7]. Dogs are used the most frequently for animal-assisted interventions (AAI), but rabbits could also be used for complementary therapy [8] as they are popular, especially among children [6,9]. Rabbits are common companion animals in many European countries [10]. Rabbit-assisted

interventions in primary schools are suitable for decreasing the anxiety levels of children, which may increase the education efficiency of teachers [11]. The body language of the rabbits is easy to comprehend because it is straightforward to separate aggressive rabbits from curious and friendly ones. By the position of their ears, their momentary mood can be determined. They are stressed when they keep their ears back, curious when their ears are forward and alert when they are in the up position. Nervousness or aggressiveness is demonstrated when they turn to face, their ears are halfway down, and they often thump with their back foot [12].

Rabbits appear as amiable figures in children's literature that generate positive feelings in pupils [8]. They have been used as therapy animals in hospitals, nursing homes and veterans' homes, social institutions, kindergartens and schools [6,7].

1.1. Stress Induced by Animal-Assisted Interventions

According to the definition of the American Veterinary Medical Association, the bond between human and animal is a "mutually beneficial relationship and essential to the wellbeing of both". However, the well-being of the animal gets less importance compared to that of the human's [13]. Fear arises when there is a feeling of danger and causes behaviour changes [14]. In case of sustained fear, chronic stress develops that deteriorates animal welfare [15].

The therapy and assistance animals have to cope with the cumulate stress caused by the interventions. The owner of the rabbit and professional participants of the AAI must recognise the warning signs of the exhaustion of the animals and be able to intervene. In the case of a stressful situation that the rabbit is not able to handle, a prompt reaction is required by the rabbit's trainer/owner, which may result in the suspension of the intervention. It is necessary to have an exact plan for each intervention that includes regular breaks between the sessions of the therapy. This provides the possibility for the animal to rest and fully recover [14]. Irrespective of the species of the animal, those people who are responsible for them (trainers, owners) must determine and monitor their stress level because the environment of AAI puts them into a high-stress situation. From the therapy point of view, the aim of animal welfare is to make a comfortable environment for rabbits in which human interaction is not unpleasant and not forced. To achieve a successful AAI program, it is essential to give importance to the animal's needs; therefore, we need to ensure recovery after interventions [15].

Changes in the behaviour of a rabbit, including hiding or unexplained aggression, unusual movements during the interventions or in their home area, such as circling within the cage or increased/decreased food intake, are the typical symptoms of fear, stress or even depression [16,17]. Pet rabbits'—similar to farmed or laboratory rabbits'—reactions were observed when a possible danger occurred (unknown person's appearance, noise, new animal). They reacted in the same way by escaping to the far end of the cage or to a hiding place, where they froze down or attacked with their claws and teeth [18–20]. Having appropriate information about the rabbits' actual status before, during and after the AAI sessions is also an indispensable part of the work [8]. To achieve adequate use of rabbits for therapy purposes, we need to fulfil the following requirements:

- The rabbits' welfare is the priority;
- It is expected that the animals feel comfortable during the sessions, which should never be forced;
- Before, during and after the interventions, we need to give time for them for acclimatization or recovery;
- Trainers/owners of the rabbits must protect them from human-animal zoonotic diseases and illnesses [21].

1.2. Preparing the Rabbits to AAI Work

Rabbits struggle less if their owners frequently make contact with them and are confident in handling their rabbits. According to Mullan et al.'s observations, rabbits with

a regular companion spent significantly more time grazing, hopping and playing [18]. According to Schepers et al.'s findings, the conditions in which pet rabbits are kept have a great effect on their welfare. Frequency of handling by the owner and the environment have an impact on the behavior of rabbits: when rabbits kept alone are picked up, they display strong resistance that indicates socialization problems, and their lifespan is reduced [22]. Inadequate socialization, territorial behavior [19], learned dominance, lack of concern, illness and physical pain, food competition, and reproductive behaviors increase the aggression of pets toward humans [19,23,24].

In many mammal species, there is a sensitive period during which the nervous system of young animals can be greatly affected. Certain specific stimuli trigger learning processes, which influence the behaviour of the individuals for the rest of their lives, i.e., there is a sensitive period for socialization. Under experimental conditions, such interventions may include handling. The taming effect of handling may have high importance in large-scale production, where working with tamer animals could be advantageous in several ways; for example, they can perform better. Handling is also important for animal therapy because an animal that is less afraid of humans from the beginning will be more suitable for therapy and easier to teach and handle; thus, better results can be achieved [25].

In rabbits, handling consists of touching and holding [26]. Hudson et al. [27] found that even minimal handling carried out in the first week of life reduces shyness. Pongrácz and Altbacker [28] concluded that repeated handling had a positive effect on the behaviour and the welfare of rabbits kept in cages. Kits will become less shy of humans if handling is carried out at times close to suckling. The first week after birth has been shown to be a sensitive period for successful handling. Bilkó et al. [26], Verga et al. [20], and Zucca et al. [29] have found similar results, i.e., handling at an early age has a significant effect on rabbits' reactivity in behaviour tests. Csatádi [25] examined the effects of early handling under laboratory and natural conditions. He used chinchilla rabbits and New Zealand white rabbits in his research. In the laboratory tests, handling, i.e., touching the kits, was carried out during the first week after birth within half an hour following suckling. At these times, the weight of the kits was typically measured, and their ears were marked: all this took about 3 to 5 min per litter. These "handled" rabbits showed less fear towards humans and became calmer in nature, which continued in their adult life. Another study was conducted to determine if the effects of handling can be specific and whether young rabbits can distinguish between two people. Handling was conducted on two litters (13-13) by two different persons for 7 days. At the age of one month, the litters were divided into two, and the same two persons conducted further handling in an approaching test. The comparison of the animals' behaviour showed that the person handling the animals did have an effect. The kits showed a preference towards the tester who had been handling them after birth, and both groups were less afraid of people compared to the group which had not been handled at all. Csatádi [25] believes that this can be most probably attributed to olfactory learning. In another experiment, Csatádi [25] examined whether the length of handling influenced its effects. In addition to the usual handling lasting for 3–5 min, 5-s-long handlings, which are actually more applicable in large-scale environments, were conducted with different groups half an hour after suckling and at least 2 h later, and finally, results were compared at the age of one month. They found that only the timing of handling had an effect, whereas its duration did not. Rabbits handled within half an hour after suckling showed less fear towards humans even when they received minimal handling. This may be explained by the fact that tameness comes down to the process of socialisation; thus, the occurrence and the timing of handling are important, but its duration is not [25].

1.3. Studies Made with Rabbits: Previous Research Results

In the spring of 2014, the University of Kaposvár, Érdligeti Primary School, Kőrösi Csoma Sándor Primary School in Érd and Bóbita Kindergarten in Törökbálint started a 6-week-long animal-assisted pilot study involving 11 dwarf rabbits. The rabbits had not

received any prior therapy training. The animals were in the kindergarten for 6 weeks, and human approach tests were recorded every second week. The relationship of the individuals with humans was tested with three different groups: children (twice a week), teachers (they were the animals' owners) and strangers (with whom the rabbits had had no prior contact). The study aimed to determine how the animals' attachment to the owner and the other persons changed over the animal-assisted therapy process. At the end of the project, the dwarf rabbits were subjected to a major stress effect when groups of 6–9 children handled them for 7–10 min. The conclusion of the study was that the carrier cages were a major stressor for the animals, so their confidentiality level decreased significantly. The strong stress effect deteriorated the previously established relationship, so the interoperability of the rabbits might have been replaced by fear, indifference or perhaps even aggression. Based on the research study, it was suggested that rabbits should receive handling for 2–3 weeks before starting therapy [30].

In another study, school-starting stress was examined using rabbits. This research involved 51 children in a regular and in an inclusive primary school. The effect of rabbits on first-formers (six- and seven-year-old children) was examined under classroom conditions. Animal-assisted development had a substantial effect on children's anxiety levels: anxiety levels decreased during the periods of the development sessions. The higher the anxiety level at the beginning, the greater the effect the animal-assisted activity had. The presence of the rabbit reduced children's anxiety levels by 9.48% in the inclusive schools and by 7.24% in the regular school. At the same time, however, the research did not provide any findings connected to the behaviour of the animals and their stress levels [11].

The objective of our present result was to analyse the behaviour and stress tolerance of dwarf rabbits in a classroom-like animal-assisted environment with the purpose of providing new results on the other participant in the therapeutic work, the rabbit. These results may be important for animal welfare reasons and also to facilitate animal-assisted work.

2. Materials and Methods

The study was conducted with 20 dwarf rabbits from a stock selected for tameness during six generations. The rabbits were the progeny of a single buck mated to three does, between the age of 8 and 11 months (mean: 9.6) and between the weight of 1.15 kg and 2.24 kg (mean: 1.579 kg). Rabbits were considered to belong to two different groups: subjects that had previously received handling during the sensitive period after birth, i.e., touching the kits and holding them within half an hour following suckling based on the methodology of Csatádi [18] ($n = 4$, Group H) and subjects without handling experience during the sensitive period right after birth ($n = 16$, Group NH).

Although according to d'Ovidio et al.'s online questionnaire ($n = 634$), the chance of aggressive behavior of pet rabbits against their owner or a strange person is significantly lower in intact males than in neutered males and does, and buck rabbits' interest in their owner is significantly higher [23], the intact males spray urine to determine their territory [19]. We did not experience the calmer temperament of the intact males, and because of their urine spraying behavior, we preferred using does for AAI works. However, in this this examination, we did not take into consideration the effect of gender to the AAI work.

2.1. Housing and Feeding

The rabbits were housed in confined sheds in cages ($95 \times 57 \times 46$ cm) for pet rabbits with a dark-colored plastic bottom covered with a metal mesh. The mesh, which was coated with corrosion-resistant paint, can be completely removed from the front of the cage for easier access, which made daily cleaning easier. Inside, the rabbit cage was roomy and included all the essential amenities: a water nozzle, a feeder, a hay container, an enclosed sleeping area and a rabbit litter box filled with wood pellets (compressed wood shavings and sawdust from untreated wood). All the accessories were made of plastic.

As for feeding, the rabbits received a complete diet, and the feed was given in pellet form in order to prevent selective eating. The pellets were composed of natural raw materials and were enriched with Florastimul (FOS), which stimulates a healthy intestinal flora. This feed does not contain a coccidiostat or other additives. The analytical data of the feed was as follows: crude protein 14.47%, crude fat 2.59%, crude fibre 14.75%, calcium 1.29%, phosphorus 0.76%, and sodium 0.18%. Additives/kg were as follows: 3a672a vitamin A 10,000 IU, 3a671 vitamin D3 1200 IU, 3a700 vitamin E (all-rac-alpha-tocopheryl acetate) 80 mg, 3b103 iron (ferrous sulphate, monohydrate) 100 mg, 3b202 iodine (calcium iodate, anhydrous) 2.10 mg, 3b405 copper (cupric sulphate, pentahydrate) 10 mg, 3b502 manganese (manganous oxide) 75 mg, 3b603 zinc (zinc oxide) 70 mg, and 3b801 selenium (sodium selenite) 0.20 mg. Digestible energy was 9.12 MJ/kg. The feed was fibre-rich to ensure good intestinal functioning. Besides the pellets, the rabbits were given ad libitum hay. Feeding was conducted twice a day: in the morning at 7 a.m. and 12 h later, at 7 p.m. The hay was continuously available from hay racks. Water was also available ad libitum from nipple drinkers. In addition, gnawing sticks and supplementary mineral blocks were provided to ensure environment enrichment.

Veterinary checks were conducted on all rabbits (feces) prior to the animal-assisted sessions to detect the possible presence of parasites (*E. cuniculi*, *Passalurus* sp.). Physical examination was also carried out, including a dental health condition check and an assessment of the appearance of *Spilopsyllus cuniculi* and *Psoroptes*. The rabbits' claws were shortened to avoid injuries. All rabbits were free from zoonosis and parasites, and all animals were vaccinated against myxomatosis and rabbit haemorrhagic disease (RHD).

2.2. Human Approach Tests

The rabbits were examined in five cases. Those animals whose behavior was passive during the interventions received higher points than those who were trying to escape, although the passive ones received lower scores than those rabbits who were friendly, curious and were looking for the company of the testing person. Clearly separating the motionless (freezing) rabbits that showed signs of fear from the rabbits that did not show any interest (staying passive during the activity) by, for example, the rabbit's ears positions and behavior was obvious to a specialist (PhD in ethology). All the tests were assisted by a PhD in ethology (M.M.) who did not have preliminary contact with the rabbits (an independent person without preconceptions about the individuals). The behavior was assessed directly.

- First, confidentiality was tested with the owner (S-B.É., a PhD student, animal husbandry engineer, pet and hobby animal breeder with a training specialization) with a test consisting of 12 tasks. (Owner Approach Test, "OAT", subject of Appendix A).
- Then, the animals' behavior and stress tolerance were tested by involving 120 children. Based on the school-teaching model, we had three 45 min long sessions in a row with 15 min long breaks between them. As part of the sessions, the children completed a simplified human approach test with the rabbits (Children Approach Test, "CAT", subject of Appendix B), the main parts of which focused on the rabbits' interest, indifference and fear. This gave us the opportunity to gain information on how the rabbit individuals reacted to repeated stress. The sessions thus yielded three test results on the relationship between children and rabbits.
- Finally, after transporting the rabbits back to their housing area, we repeated the 12-item Owner Approach Test on the rabbits with the owner. Thus, we obtained results on the extent to which the animal-assisted session affected the rabbits' confidentiality with their owners and indirectly on the level of stress.

Our hypothesis:

- There is a significant difference between the rabbits' stress toleration levels according to the handling they received during the sensitive period after birth.

- In a three-times repeated animal-assisted intervention, the rabbits are able to cooperate without significant decreases in their stress toleration if the interventions do not take longer than 20 min and we give at least 30 min retreat for them.

2.3. The Owner Approach Test (OAT)

During the test, the owner (S-B.É.) gradually approached the animals and assessed their behavior by observing their reactions. First, the owner put a hand on top of the closed rabbit cage. The owner then rattled the cage a little, opened the door and reached for the rabbit. The owner then placed a hand in the middle of the cage, waiting motionlessly to see if the rabbit approached the hand. Then the owner made physical contact with the animal, touched and stroked it and took the hand out of the cage to see if the rabbit followed the movement of the hand. After that, the owner took the rabbit out of the cage, placed it in their lap and kept stroking it. Then, 15 s later, the owner stood up with the animal in their hands and kept stroking it. Later the owner put it on the ground and observed the exploratory behavior of the rabbit. Then the owner reached towards the animal and observed whether it followed the movement of the hand. Finally, the owner put the animal back into its own cage and observed the animal's reaction through the open door, including whether it looked out of the cage or perhaps jumped out of it.

The numbers of the responses listed under each aspect (1–12) also represent the point value with a higher score increasing in line with the expected behavior. During the tasks, the contact with the rabbit increased, and the PhD (in ethology) assistant scored the rabbits by choosing the most appropriate answers to the aspect. For example, in the first task, the assistant directly examined the rabbit's reaction while the owner kept 1 m distance from the front of the rabbit's cage. The rabbit got "one" point if it ran to the far end of the cage and showed signs of fear and "two" points if its behavior was uncertain and if it was keeping off. The rabbit got "three" points when it did not show any interest (staying passive during the activity) and "four" points if it was looking around, paying attention, keeping its ears up and walking around the cage curiously. Finally, the rabbit got the maximum of "five" points when it was coming closer to the owner and walking around curiously. The OAT test is the subject of Appendix A. In parallel, the animal's response latency was also measured. For each activity, the behavioral response was observed for 15 s except for point 12, where the animal had 30 s to jump out of the cage through the open cage door. The rabbits received additional 'bonus' points depending on their response time measured in seconds (latency) to the situations to emphasize the importance of cooperating easily and quickly. The evaluation of the response time in situations 1–6 and 10–12 are shown in Table 1.

Table 1. The evaluation of the response time in situations 1–6 and 10–12.

Seconds	Bonus
0–3	1
4–6	0.8
7–9	0.6
10–12	0.4
13–15	0.2

The highest potential score during the test was 64.

2.4. The Children Approach Test (CAT)

The study was conducted twice with 10 rabbits in each case in the children's summer camp organized by the Family and Child Protection Service in Várpalota in 2021. The supervisor of the animals was the owner of the rabbits (first writer—PhD student in ethology). The directly assisted test tasks were completed by the third author (PhD in ethology).

With the camp application form, the parents received a detailed written description of the planned animal-assisted interventions and gave their permission to let their child par-

ticipate in the sessions and be in photos with free usage by their signature. The documents are kept in the Family and Child Protection Service in Várpalota and held for 5 years.

From the site of the animals to the children's summer camp, the rabbits were transported within 45 min by an air-conditioned minibus. The rabbits were delivered in individual plastic carrier cages with a metal gate. The size of the carrier was $32.5 \times 48 \times 29$ cm, with 1×9.3 blowholes on the sides of the cage. The metal gate of the carrier was 23×23.5 cm with 2.5×2.7 cm unit cells and 1×9.3 blowholes on the sides. Nipple drinkers were refilled with fresh water and fixed on each cage. Hay was also available ad libitum from hay racks.

As the conveyance itself and the use of the carrier cages caused stress to the rabbits [9], the animals were placed in the study room to rest for an hour after the transport before the sessions began while each rabbit was placed individually in cages ($95 \times 57 \times 46$ cm) for pet rabbits. Water was available ad libitum from nipple drinkers.

There were 60 children in the camp for each test, and they were divided into three groups, so 3 times 20 children participated in the daily sessions. The purpose of the AAI to the children was to educate them on how to be responsible for animals and give basic information about rabbits' needs, housing, feeding and health care. Each rabbit was handled by two children at a time, assisted by a teacher/camp counsellor (Figure 1) who was previously educated on basic rabbit behaviors, especially signs of fear and aggression and proper handling, including how to pick up and hold the animal, in order to be able to intercede if needed.



Figure 1. Children Approach Test—session 1, rabbits' emplacement.

Based on the Hungarian school-teaching model, the sessions were 45 min long, followed by a 15 min break [source of law: 20/2012. EMMI decree 16. § (2)]. During the break, the groups of children were changed, and the rabbits were allowed to rest in their cages. As an introduction to the session, the children attended a 20-min-long lecture on the basics of animal welfare and animal keeping. Afterwards, the rabbits were touched and handled by the children (which increased levels of stress). All the rabbits were handled three times in a day as the sessions followed each other. Altogether, the rabbits were handled by six children and one camp assistant.

As a part of the session, the children conducted a simplified test of 8 tasks with the rabbits, the main parts of which focused on the animals' interest, indifference and fear. Three sets of two children handled one rabbit at a time (plus one assisting teacher); thus, three sets of surveys were made about each individual.

During the test, the children gradually approached the animals and assessed their behavior by observing their reactions. First, they placed their hands on top of the closed rabbit cage, then rattled the cage slightly, opened the door and reached for the rabbit. Then they stroked the animal while it was still in the cage, and both children sat down on the floor opposite each other. Then the person assisting the examination took the rabbit out and put it down near the children in the way shown in Figure 2a. The children then picked up the rabbit into their arms and stood up with it (Figure 2b). Then, they both stroked it and finally put it back into the cage.

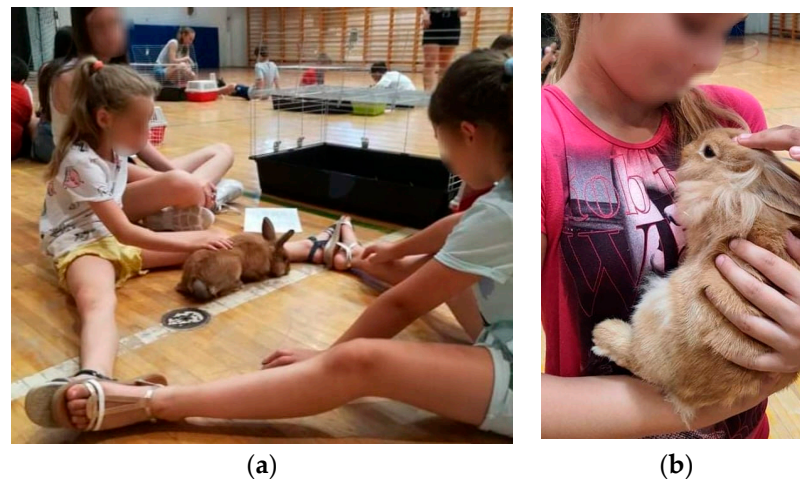


Figure 2. CAT (a) rabbits on the floor and (b) rabbits in children's hands.

The 8 tasks took 20 min in total to complete. A maximum of 4 points could be given, with the lowest score given to an animal showing signs of fear (startled, shying away, fleeing), 2 points given when the rabbit remained indifferent, 3 points when it showed some interest and 4 points when it made contact with the children or tolerated the task calmly. This gave a possible maximum score of 32 points for one individual.

2.5. Statistical Analysis

Regarding the Owner Approach Test (OAT) and the Children Approach Test (CAT), the effects of handling (treated with handling vs. control) and the number of measurements (OAT: first and second; CAT: first, second and third) on the rabbits' score were analyzed using generalized linear model (GLM) analysis taking into account that the same rabbits were evaluated repeatedly. Statistical analysis was performed using SAS 9.4 software using the PROC MIXED procedure.

3. Results

3.1. The Rabbits' Behavior According to the Owner Approach Test

The confidentiality of the rabbits against their owner decreased by the impact of the children's interventions. The difference between the results of the OAT made before and after the intervention is significant ($p = 0.0002$), and the results are presented in Table 2. There is a substantial difference between the individuals according to the trend of the data. While rabbits 8 (NH) and 11 (NH) reached higher scores on the OAT made after the animal-assisted interventions, rabbits 2 (H) and 4 (H) scores decreased by less than a point. Most of the rabbits demonstrated decreased confidentiality against their owner. This dynamical deficit appeared in rabbits 5 (NH), 6 (NH), 10 (NH), 14 (NH), and 19 (NH) by almost 5%, while in rabbits 16 (NH) and 18 (NH), this reduction was above 10%. Rabbit 20 (NH)'s decrease was nearly 10%.

The totalized means of the approach tests made before and after the interventions draw a negative distinction between the scores (Figure 3A). The difference is significant ($p = 0.0035$). The handled (H) rabbits against the non-handled (NH) ones reached higher scores on both of the owner approach tests that were made before and after the AAI (Figure 3B).

Table 2. The total scores of the 20 rabbits before and after AAI.

Rabbit Number	Total Score		Difference
	Before AAI	After AAI	
1(H)	54.6	52	−2.6
2(H)	55	54.8	−0.2
3(H)	56.8	55.4	−1.4
4(H)	52.8	52.8	0
5(NH)	52.6	47.8	−4.8
6(NH)	52.8	47.2	−5.6
7(NH)	51.6	48.8	−2.8
8(NH)	48.4	49	0.6
9(NH)	42.2	38.6	−3.6
10(NH)	38.8	34.2	−4.6
11(NH)	46.2	47.2	1
12(NH)	39.4	37	−2.4
13(NH)	45	42.4	−2.6
14(NH)	47.2	41	−6.2
15(NH)	45	40.4	−4.6
16(NH)	41	28.8	−12.2
17(NH)	35.6	34.2	−1.4
18(NH)	39.8	26.2	−13.6
19(NH)	41.4	34.6	−6.8
20(NH)	47.2	37.8	−9.4

H = handled, NH = non-handled. H = these rabbits were handled during the kits' sensitive period so they were socialized to human touch; NH = these rabbits did not receive any training during their sensitive period.

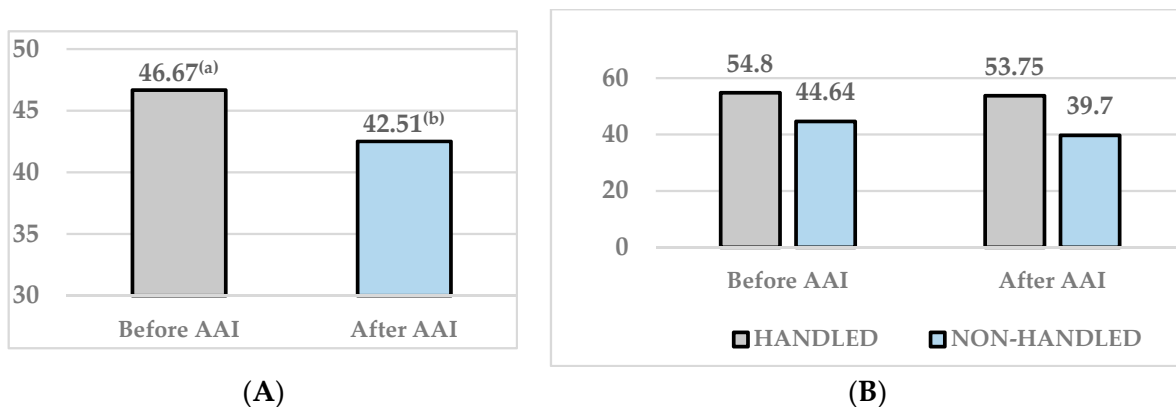


Figure 3. Owner Approach Test results: made before and after animal-assisted interventions ($p = 0.0002$). (A) Total mean and (B) H and NH rabbits mean. In (A), means with different letter marks significantly diverge from each other ($p < 0.05$).

Before the AAI, the mean of the handled rabbits' scores on the owner approach test achieved 54.8 points, while after the AAI, it reached 53.75 points. The AAI caused a 1.92% degradation in handled rabbits' confidentiality level, which is not significant ($p = 0.18$).

The NH rabbits' total means according to the owner approach test before the AAI reached a score of 44.64. In itself, this score is already 18.55% lower compared to the handled rabbits' scores. The repeated owner approach test that was made after the AAI shows a 1.98% degradation in the handled rabbits' confidentiality level. The mean of the NH group at the repeated owner approach test was 39.7 points, which is an 11.07% degradation compared to the results of the tests made before the AAI. There is a significant difference between the H and NH group of the rabbits' Owner Approach Test results made after the AAI ($p = 0.0002$). Handled rabbits achieved a 26.14% higher score on the OAT test made after the AAI than the NH ones.

3.2. Rabbits' Behavior According to the Children Approach Test

The behavior changes of the individual rabbits during the animal-assisted interventions are presented in Table 3.

Table 3. Results of the Children Approach Test in AAIs.

Rabbit Number	Total Score			Differences between 1st and 3rd AAI
	1st AAI	2nd AAI	3rd AAI	
1(H)	29	27	20	−9
2(H)	27	26	25	−2
3(H)	28	26	23	−5
4(H)	25	22	16	−9
5(NH)	23	21	22	−1
6(NH)	22	20	19	−3
7(NH)	27	25	21	−6
8(NH)	22	20	18	−4
9(NH)	20	9	16	−4
10(NH)	22	25	29	7
11(NH)	25	23	20	−5
12(NH)	20	17	23	3
13(NH)	25	25	20	−5
14(NH)	20	23	25	5
15(NH)	23	18	19	−4
16(NH)	23	20	14	−9
17(NH)	23	23	20	−3
18(NH)	19	19	19	0
19(NH)	26	22	22	−4
20(NH)	17	26	15	−2

H = handled, NH = non-handled.

According to the data from the repeated interventions, the rabbits showed less interest in children. During the first intervention, the animals took part in the exercises without any shyness; their curiosity was obvious. They cooperated in most of the tasks. Rabbits were willing to get closer to children while they were in the cage, and when they were taken out and children touched them, their behavior was calm. Their ears were in a watchful position, and they threw up their heads and frequently looked around. When the rabbits were down on the floor, allowing them to move freely, after a short acclimatization period, they tended to discover the environment and took some leaps around the children. None of them were frightened or froze down.

Figure 4A shows the total mean of all rabbits in the 1st, 2nd and 3rd interventions. Between the first and third intervention, a significant difference of $p = 0.02$ was detected. Figure 4B presents the mean of the rabbits in the three sessions divided into two groups: handled and non-handled. As Figure 4A shows, the mean on the Children Approach Test for the first session was 23.3 points. However, the difference between the handled and the non-handled rabbits' mean (Figure 4B) is significant ($p = 0.0114$). The handled group reached 27.25 points out of the maximum of 32, while the NH ones mean result reached 22.31 points, which is 18.13% worse compared to the H group. As Figure 4B presents, the handled rabbits' confidentiality during the first and second children interventions did not show such a deterioration as appeared by the third CAT. The decrease is not significant ($p = 0.33$) between the first two interventions, but it is between the second and the third one ($p = 0.04$). In contrast, the NH rabbits' confidentiality was 18.2% lower than the H rabbits at the first CAT, and a similar difference was detected by the second CAT (16.84%). For the third intervention, the difference between the handled and non-handled groups dropped to 4.15%, but the handled group still reached the higher scores.

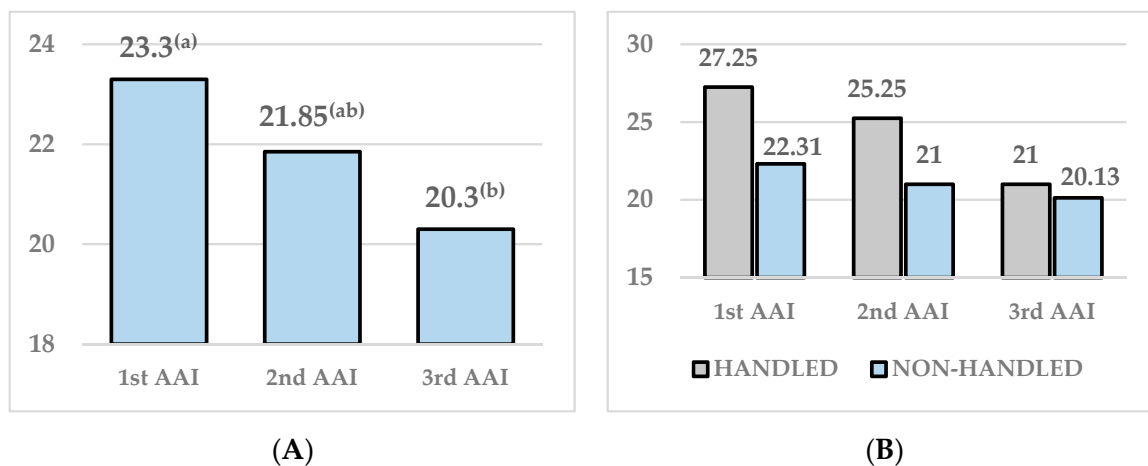


Figure 4. Children Approach Test results over the three sessions ($p = 0.02$; difference was detected between the first and third AAI). (A) Total mean, (B) handled and non-handled rabbits' means. In (A), means with different letter marks significantly diverge from each other ($p < 0.05$).

4. Discussion

The most frequently used species for AAIs is dogs, but for complementary therapy, rabbits could also be used [8] as they are popular, especially among children [6,9], and in many European countries, rabbits are common companion animals [10]. Rabbits appear in children's literature as benevolent figures that eventuate positive feelings in pupils [8]. Molnár (et al.) proved that the anxiety level of primary school children can be decreased by rabbit-assisted interventions, which may have positive effects on the education efficiency of teachers [11]. Rabbits have been used for therapy purposes in hospitals, nursing homes and veterans' homes, social institutions, kindergartens and schools [6,7], although only a limited number of animal species and individuals are suitable for therapy. Therefore, preliminary knowledge of the animal's behaviour to ensure the safety of the person involved in the therapy and the well-being of the animals are all very important [6]. Handling the rabbits during their early age period has a positive effect on their stress toleration level [25,26]. The body language of the rabbits is easy to comprehend because it is clear to separate the aggressive rabbits from the curious and friendly ones. By the position of their ears, their momentary mood can be determined [12].

Our first hypothesis was that there is a significant difference between the rabbits' stress toleration levels according to the handling they underwent during the sensitive period after birth. Our study confirms this statement. The handled rabbits were willing to cooperate during the tasks in both human approach tests better than the non-handled ones. Their confidentiality was higher; they showed fewer signs of fear and felt more calm through the AAI.

Our second hypothesis was that in a three-times repeated animal-assisted intervention, the rabbits would be able to cooperate without a significant decrease in their stress toleration if the interventions did not take longer than 20 min and we provided at least 30 min retreat for them. Our study does not confirm the statement. According to the school teaching model, a session where children were present lasted for 45 min, which was followed by a 15-min-long break. During the sessions, children dealt with the rabbits for 20 min with increasing intensity.

Although the average scores of the rabbits did not decrease significantly (mean: 1st AAI: 24.78, 2nd AAI: 23.12, 3rd AAI: 20.56), there is a difference between the results of the handled and the non-handled groups of rabbits. Means were as follows: 1st AAI/handled: 27.25, 1st AAI/non-handled: 22.31, 2nd AAI/handled: 25.25, and 2nd AAI/non-handled: 21. Furthermore, there was a significant decrease in the third intervention in the scores of the handled rabbits that reached 21 points ($p = 0.04$). Based on these findings, we cannot accept our second hypothesis. However, handling plays a very important role in the stress

tolerance of rabbits because these animals were more cooperative than the non-handled ones not only at their first intervention but also in their second intervention. Only in their third session did they show a considerable decrease in cooperation.

The importance of having information about the stress toleration capacity of the rabbits during the sessions and the effect of repeated sessions is reasonable in order to get proper knowledge about the rabbit-assisted intervention's methodology, avoid exhaustion of the animals and ensure their mental and physical health. The limitation of our study is that determining the stress level of the rabbits by behavior examination is not exact, although it gives us clear information, and the tests were fulfilled by a specialist (PhD in ethology). To have accurate data parallel with the behavior examination hormone of control is reasonable to determine. Monitoring stress hormone metabolites with non-invasive tools is appropriate for animal welfare assessment. In case the faecal cortisol and corticosterone metabolites are measured in the same way, their levels can be compared according to different stress situations [31]. Our tests could be verified by defining cortisol metabolites' hormone levels. We suggest extending the examination by involving higher numbers of handled rabbits.

5. Conclusions

The stress tolerance of rabbits receiving handling exceeds that of those that were not handled previously. Thus, rabbits that are intended to be used for animal-assisted interventions are strongly recommended to undergo handling during their sensitive period after birth, as the rabbits receiving handling show measurably higher interest and cooperation. By handling the rabbits, the possibility of children's injuries can be reduced (the animal suddenly jumping in children's hands, causing scratches), and it is also important with regard to animal welfare. As a result of the higher stress tolerance during animal-assisted sessions, the risk of animal exhaustion is lower, and their behavior is calmer even after an event of stress. Thus, we can conclude that handling plays a very important role in stress tolerance.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Owner Approach Test (OAT)

Rabbit number: _____

BEFORE/AFTER (underline) Animal-Assisted Intervention

1. The rabbit's reaction while the owner is keeping 1-meter distance from the front of the rabbit's cage

1. The rabbit is running to the far end of the cage and showing signs of fear.
2. The rabbit is uncertain and keeping off.
3. The rabbit does not show any interest (staying passive during the activity).
4. The rabbit is looking around, paying attention with its ears up and walking around the cage curiously.
5. The rabbit is coming closer to the owner and walking around curiously.

_____s

2. The rabbit's reaction while the owner is putting one hand on the top of the cage

1. The rabbit is running to the far end of the cage and showing signs of fear.
2. The rabbit is uncertain and keeping off.
3. The rabbit does not show any interest (staying passive during the activity).
4. The rabbit is looking around, paying attention with its ears up and walking around the cage curiously.
5. The rabbit is coming closer to the owner and walking around curiously.

_____s

3. The rabbit's reaction while the owner is making some noise with fingers on the cage

1. The rabbit is running to the far end of the cage and showing signs of fear.
2. The rabbit is uncertain and keeping off.
3. The rabbit does not show any interest (staying passive during the activity).
4. The rabbit is looking around, paying attention with its ears up and walking around the cage curiously.
5. The rabbit is coming closer to the owner's hand, physically touching and smelling it and curious about it.

_____s

4. The rabbit's reaction while the owner is opening the cage and putting one hand in the middle of the cage and staying motionless

1. The rabbit is running to the far end of the cage and showing signs of fear.
2. The rabbit is uncertain and keeping off.
3. The rabbit does not show any interest (staying passive during the activity).
4. The rabbit is looking around, paying attention with its ears up and walking around the cage curiously.
5. The rabbit is coming closer to the owner's hand, physically touching and smelling it and curious about it.

_____s

5. The rabbit's reaction while the owner is stroking it

1. The rabbit is running to the far end of the cage, and when the owner's hand approaches again, the rabbit is trying to escape and running away.
2. The rabbit is running to the far end of the cage, and when the owner's hand approaches again, the rabbit is freezing down and lets the owner stroke it.
3. At first, the rabbit lets the owner stroke it for at least 5 s but afterward, it is trying to escape and running away.
4. The rabbit lets the owner stroke it, but it does not initiate the activity.
5. The rabbit lets the owner stroke it and shows interest by keeping its ears up, looking forward to the owner's hand by smelling it and physically touching it by its nose.

_____s

6. The rabbit's reaction while the owner is moving his hand in the cage. Is the rabbit following the owner's hand?

1. The rabbit is running to the far end of the cage and showing signs of fear.
2. The rabbit is staying motionless (at least for 5 s) and then running to the far end of the cage.
3. The rabbit does not show any interest (staying passive during the activity).
4. The rabbit is looking around, paying attention with its ears up and walking around the cage curiously.

5. The rabbit is following the owner's hand, showing interest and keeping its ears up.
_____s
- 7. The owner is taking out the rabbit from the cage, sitting on a chair and stroking the rabbit on her lap. What is the rabbit's reaction?**
1. The rabbit is immediately trying to escape.
 2. The rabbit lets the owner stroke (at least for 5 s), but then it tries to escape.
 3. The rabbit lets the owner stroke it.
- _____s
- 8. The rabbit's reaction while the owner is standing up and holding the rabbit in her arms.**
1. The rabbit is immediately trying to escape.
 2. The rabbit lets the owner stroke it (at least for 5 s), but then it tries to escape.
 3. The rabbit lets the owner stroke it.
- _____s
- 9. The rabbit's reaction while the owner stays standing up, holding it in his arms and stroking it.**
1. The rabbit is immediately trying to escape.
 2. The rabbit lets the owner stroke it (at least for 5 s), but then it tries to escape.
 3. The rabbit lets the owner stroke it.
- _____s
- 10. The rabbit's reaction while the owner is putting the rabbit down on the floor, where she is stroking it.**
1. The rabbit is immediately trying to escape and showing signs of fear.
 2. The rabbit is staying motionless (freezing down) and showing signs of fear.
 3. The rabbit lets the owner stroke it (at least for 5 s) but then tries to escape.
 4. The rabbit lets the owner stroke it and shows interest, keeping its ears up, smelling and looking around curiously or discovering the environment.
- _____s
- 11. The rabbit is on the floor, and the owner is moving her hand (getting closer to the rabbit, then pulling away her hand). Does the rabbit follow the owner's hand?**
1. The rabbit is immediately trying to escape and showing signs of fear.
 2. The rabbit is staying motionless (freezing down) and showing signs of fear.
 3. The rabbit does not show any interest (staying passive during the activity).
 4. The rabbit is discovering the environment but is not paying any attention to the owner's hand, keeping its ears up and looking around.
 5. The rabbit shows interest, coming closer to the owner's hand and following it.
- _____s
- 12. The owner puts the rabbit back in the cage, leaves the cage open and keeps 1 meter of distance from the front of the rabbit's cage. What is the rabbit's reaction?**
1. The rabbit is running to the far end of the cage and showing signs of fear.
 2. The rabbit is staying motionless (freezing down) and showing signs of fear.
 3. The rabbit is coming closer to the gate of the cage, sticking out its head, keeping its ears up and looking around.
 4. The rabbit is coming closer to the gate of the cage, keeping its ears up, jumping out and looking around.
- _____s

Appendix B

Children Approach Test (CAT)

Rabbit number: _____

FIRST/SECOND/THIRD (underline) Animal-Assisted Intervention

1. Put one of your hands on the top of the rabbit's cage. What is the rabbit doing?

1. The rabbit seems to be frightened and is running to the far end of the cage.
2. The rabbit does not show any interest and is staying moveless.

3. The rabbit is looking around, its ears up and walking around the cage curiously.
4. The rabbit is coming closer to my hands and smelling it.
- 2. Your hand is still on the top of the rabbit's cage. Make a little noise with your fingers on it. What is the rabbit doing?**
 1. The rabbit seems to be frightened and is running to the far end of the cage.
 2. The rabbit does not show any interest and is staying motionless.
 3. The rabbit is looking around, its ears up, and it is walking around the cage curiously
 4. The rabbit is coming closer to my hands and smelling them.
- 3. Open the rabbit's cage and put one hand in the middle of it. BUT DO NOT MOVE YOUR HAND! What is the rabbit doing?**
 1. The rabbit seems to be frightened and is running to the far end of the cage.
 2. The rabbit does not show any interest and is staying motionless.
 3. The rabbit is looking around, its ears up, and it is walking around the cage curiously
 4. The rabbit is coming closer to my hands and smelling it.
- 4. Carefully try to touch the rabbit and start to stroke it. What is the rabbit doing?**
 1. When my hand is getting closer to the rabbit, it is trying to escape and running away.
 2. When my hand is getting closer to the rabbit, it is running to the far end of the cage, but I can stroke it.
 3. The rabbit let me stroke it, and it is not running away.
 4. The rabbit likes it and shows interest by keeping its ears up. It is following my hand.
- 5. Sit on the floor with your classmate face to face and stretch your legs. Wait for a little while your teacher puts the rabbit in the middle. What is the rabbit doing?**
 1. The rabbit is immediately trying to escape.
 2. The rabbit is trying to escape, but after a few seconds, it lets us pet it.
 3. The rabbit lets us stroke it, but after a few seconds, it is trying to escape.
 4. The rabbit is staying calm and lets us stroke it.
- 6. Your teacher picks the rabbit up from the floor. Stand up and wait for a little while your teacher puts the rabbit into your hands. What is the rabbit doing?**
 1. The rabbit is immediately trying to escape.
 2. The rabbit is trying to escape, but after a few seconds, it lets me hold it in my hands.
 3. The rabbit lets me stroke it in my hands, but after a few seconds, it is trying to escape.
 4. The rabbit is staying calm and lets me stroke it.
- 7. Stroke the rabbit with your classmate together, and then put the rabbit into your classmate's hands with the help of your teacher. What is the rabbit doing?**
 1. The rabbit is immediately trying to escape.
 2. The rabbit is trying to escape, but after a few seconds, it lets us pet it.
 3. First, the rabbit lets us stroke it, but after a few seconds, it is trying to escape.
 4. The rabbit is staying calm and lets us stroke it.
- 8. With the help of your teacher, put the rabbit back in the cage and leave its gate open. What is the rabbit doing?**
 1. The rabbit is frightened. It is immediately running to the far end of the cage, where it is motionless.
 2. The rabbit is staying uninterested and is not coming closer to the gate.
 3. The rabbit is coming closer to the gate, looking out of the cage with its ears up.
 4. The rabbit is coming closer to the gate, looking out and jumping out of the cage.

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