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TRANSFUSION THERAPY IN CALVES: AN EFFICIENCY ASSESSMENT

EVALUAREA EFICIENȚEI TRANSFUZIONALE LA VIȚEI

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ABSTRACT

Blood transfusion therapy in bovine field practice is rarely used because its' limited efficiency. The aim of this study is the assessment of the efficiency of blood transfusion in cattle, on haematological and biochemical parameters using an experimental protocol. Nine healthy male Holstein calves 5 months old were subject to an anaemia induction protocol, then randomly distributed into three groups, control, autotransfusion and heterotransfusion group. The compatibility was decided using the Crossmatch test. Contrary to control group, the other two groups, receiving blood replacement therapy, exhibit a fast improvement by approximately 20% ($p<0.01$). One day latter, the haematological parameters decreased by 10% in heterotransfusion group, in two days the values were similar to those of control group. Autotransfusion group had a better evolution ($p<0.01$), however, five day after, the haematological parameters relapse by approximately 10% until the regenerative response became established. Blood transfusion was able to maintain the plasma albumin levels, and post transfusional haemolysis has no obvious impact on plasma biochemistry. In conclusion, when using Crossmatch test, blood transfusion in cattle had approximately two days efficiency.

Keywords: haematology, anaemia, cattle

REZUMAT

Terapia transfuzională la bovine este rar utilizată datorită eficienței sale limitate. Scopul acestui studiu este evaluarea eficienței transfuziei de sânge la bovine, asupra parametrilor hematologici și biochimici. Au fost utilizați nouă viței Holstein, masculi sănătoși în vârstă de 5 luni. Animalele au fost supuse unui protocol de inducere a anemiei, apoi distribuiți în mod aleatoriu în trei grupuri, grup martor, autotransfuzie și heterotransfuzie. Compatibilitatea a fost stabilită folosind testul Crossmatch. Spre deosebire de grupul martor, celelalte două grupuri, care au beneficiat de substituție sanguină, prezintă o îmbunătățire rapidă de aproximativ 20% ($p<0,01$). După o zi, parametrii hematologici au scăzut cu 10% în grupul heterotransfuzional, iar după două zile valorile au fost similare cu cele ale grupului martor. Grupul cu autotransfuzie a avut o evoluție mai bună ($p<0,01$), cu toate acestea, la cinci zile post-terapeutic, parametrii hematologici au scăzut cu aproximativ 10% și s-au ameliorat doar după apariția răspunsului regenerativ medular. Transfuzia de sânge a reușit să mențină nivelurile de albumină plasmatică, iar hemoliza post-transfuzională nu are un impact evident asupra biochimiei plasmatice. În concluzie, prin utilizarea testului Crossmatch, transfuzia de sânge la bovine a avut o eficiență de aproximativ două zile.

Cuvinte cheie: hematologie, anemie, bovine

Blood transfusion represents an emergency therapy which consists in administering blood or any of its products (1). In cattle, the transfusion indications include severe anaemia, haemorrhage with hypovolemia, haemostasis disorders, restauration of plasmatic protein level and in failure of passive colostrum immunoglobulin transfer in calves).

Severe anaemia in cattle can be caused by a wide spectrum of factors and includes parasites as *Babesia spp*. and *Mycoplasma spp*., viruses (viral diarrhoea virus) and bacteria (*E. coli*) (2, 6, 14).

While in dogs and cats blood transfusion therapy became a common procedure (4, 5), including in Romania (2, 8, 9, 12), in cattle, blood transfusion has very limited efficiency, because usually the donors Red Blood Cells (RBCs) do no remain more than few days in the circulatory system. One obvious reason of this accelerate removal of RBCs is the intricate blood group system found in those animals, which make finding compatible blood really challenging (1). Practically, the only way of establishing the blood compatibility, in farm condition is the crossmatch test; crossmatch test is able detect the incompatibilities between donor and recipient, in absence of blood typing. However, in many cases it fails to prevent all immunologic transfusion reactions including the delayed-type haemolytic transfusion reactions because it is not sensitive enough to detect low titter of antibodies (3). In fact,

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little is known about the mechanisms responsible for the very short life span of RBCs after transfusion.

The study aimed at in vivo assessment of the efficiency of blood transfusion on haematological and biochemical parameters using an experimental protocol, in controlled condition. The haematological parameters of animals subjected to compatible blood transfusion were compared not only with animals not receiving therapy, but also with animals subjected to autotransfusion. The idea beneath this protocol was to elucidated whether the limitation of life span of donor's RBCs into the recipient's vascular system is mediated by an autoimmune mechanism only, or other mechanisms are also responsible for their accelerate removal.

MATERIALS AND METHODS

Calves and farm of study

Nine healthy male Holstein calves, 5 months old (153 ± 4 kg), were selected and randomly distributed into three groups:autotransfusion group, heterotransfusion group, and control group.

The study was performed with the approval of the Bioethics and Research Ethics Comity of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (authorisation no 81 / 21.11.2017) and the consent of the farm owner. This clinical study was performed following the widely accepted veterinary practice guidelines, all animals received the adequate therapy provided by qualified veterinarians. All subjects were fully recovered at the end of the study and return into the normal farm system.

The research project was performed in a dairy cattle farm from Alba County, Romania.

Anaemia Induction Protocol (AIP)

AIP consisted in the extraction of 38% of the total circulating blood volume using a 12 gauge 80-mm catheter (Vygon, Ecouen, France), the catheter was maintained into the jugular vein up to the end of the study. The AIP was performed in two stages in order to obtain a moderate anaemia and avoiding secondary hypovolemia effects, first 20% of total circulating blood were extracted without any fluid compensation (10) and the rest of were collected by simultaneously administering intravenous fluids through saphenous catheter, in same quantities (4). Blood was collected in 450 ml blood transfusion bags with Citrate Phosphate Dextrose (CPDA) solution (Lotus Global, London, United Kingdom), being preserved in transfusion bags for 24 hours at 4°C.

Transfusion therapy protocol

At the beginning of the study, calves go through a complete clinical exam followed by Complete Blood Count (CBC) and plasma biochemistry.

In the second day, the animals included in the study were subjected to AIP in isovolemic conditions (11). Following the AIP, in the next day one group (n =3) received its own blood - autotransfusion group, other group received compatible blood from other individual – heterotransfusion group, while the other received the same volume of replacement fluid.

A three-way perfusion switch was attached to the saphenous catheter; saline solution 0.9% (Helvetica Profarm S.A., Timisoara, Romania) and a mixture of Duphalyte (Phizer, Kent, United Kingdom) and Calcium Borogluconate 30% (S.C. Pasteur, Filipesti, Romania) were attached to the switch.

Before transfusion, each blood bag was gently homogenized and bought at 36-37 °C in warm water bath, 10 minutes before administration.The bags were connected to the jugular catheter through disposable transfusion set with filter. Each animal received 18 ml/ Kg b.w with a rate of 20-40 ml/kg/h without exceeding one litre per hour (7); this way the PCV increased to maximum 10 % to avoid circulatory overload (1, 3, 10). During the entire protocol, each calf was carefully monitored by checking heart rate, respiration and general attitude.

Blood compatibility testing

The blood compatibility was established using both, major and minor crossmatch test.

The crossmatch test is a hemagglutination test; the major crossmatch detects the compatibility between donor's RBCs and the recipient plasma, while the minor crossmatch detects the compatibility between donor's plasma and the recipient RBCs. The test is known to be less sensitive in case of cattle because of low ability of their RBCs to agglutinate (1, 3). However, they are sensitive to haemolysis.

The test was performed using fresh, EDTA-anticoagulated blood. Following centrifugation step (5 minutes at 1000 G and 20°C), the plasma was collected in separate tubes. RBCs were washed three times with phosphate buffer solution (PBS) 0.1M (Sigma) (5 minutes at 1000 G and 20°C). After third washing step, the RBCs were re-suspended in PBS in a concentration of 5 %. For the major crossmatch test, we mixed into an Eppendorf tube, 2 drops of potential recipient plasma with a drop of donor RBCs, and for the minor crossmatch test plasma of the donor and recipients RBCs. One drop of Complement sera from rabbit was added in each tube (One Lambda, Meerbusch, Germany). The control consisted in plasma and RBCs originated from the same individual. The mixture was incubated for 15 minutes at 37°C, then, it was centrifuged for 15 seconds at 1000 G. The samples negative for haemolysis, were further re-suspended and examined microscopically for agglutination (1).

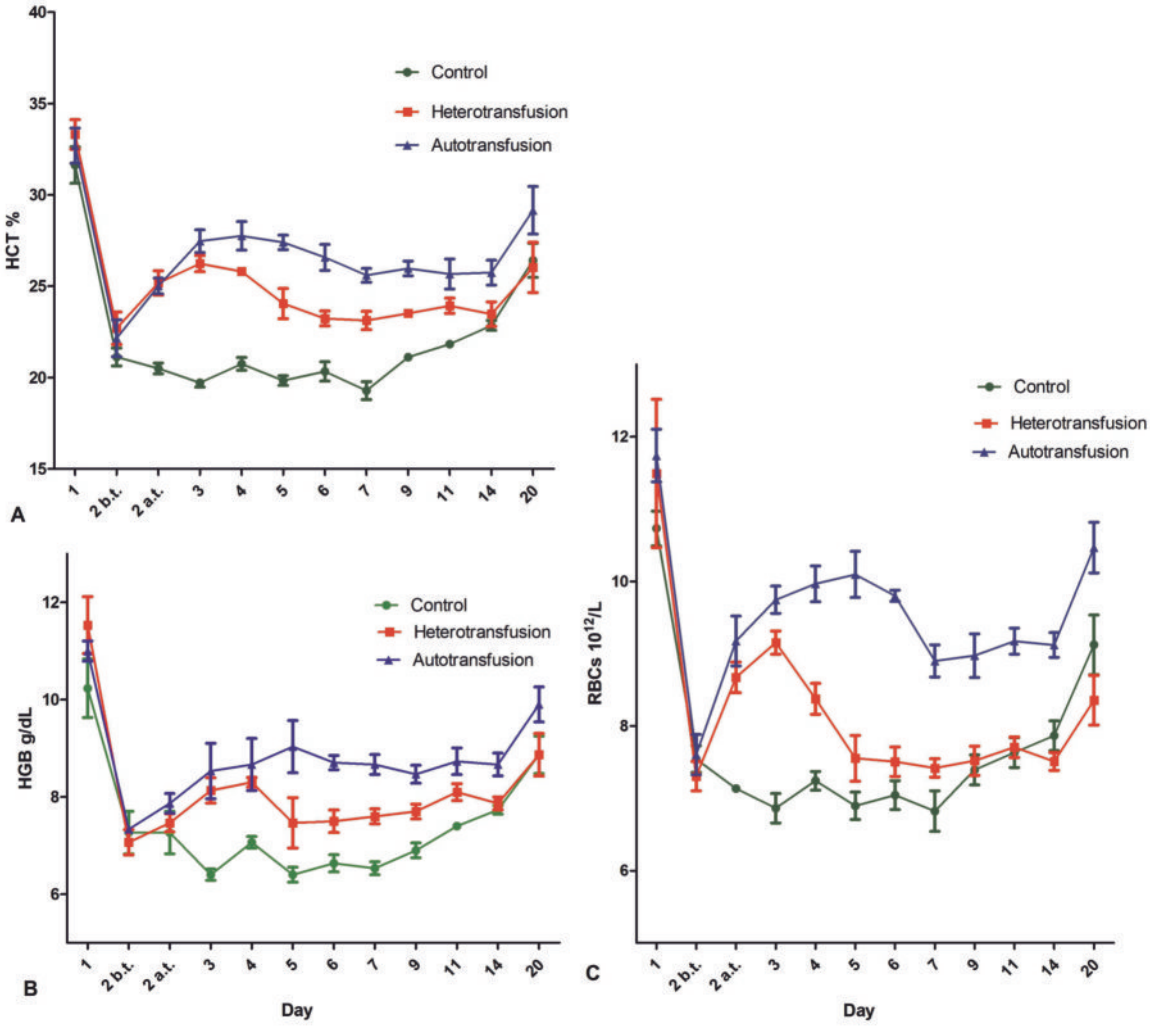

Fig. 1. The efficacy of transfusion therapy on haematocrit (HCT %) (A), haemoglobin (HGB g/dL) (B) and Red Blood Cells count (RBCs 10^{12}/L) (C). (mean ± SD) (n=3; two-way ANOVA followed by Bonferroni's post-test)

Blood samples, complete blood count and serum chemistry

Blood samples were collected from all individuals on day 1 to create the experimental groups, day 2 (before and after AIP) day 3 (before transfusion), days 3, 4, 5, 6, 7, 9, 11, 14, and 20. The Complete Blood Count (CBC) was investigated using fresh EDTA-anticoagulated blood by a Coulter based haematology analyser (Abacus Junior 5 Diff, automatic analyser, Diatron Messtechnik, Budapest, Hungary). Serum chemistry was performed using blood samples collected on clot activator. Blood samples were allowed to clot, and then centrifuged at 1500 G for 5 minutes at 20°C. Serum was immediately removed, stored at -20°C and thawed just before use for the biochemical analysis with a screen point semi-automatic analyser (STAT – FAX 1904 Plus, Global Medical Instrumentation, Ramsey, Minnesota, USA), using special kits for plasma biochemistry (Diagnosticum Zrt, Budapest, Hungary) according to the producer specifications.

Statistical analysis

All data were reported as Mean ± SD. To assume Gaussian distribution normality distribution was checked by Shapiro-Wilk normally test, then normally distributed values were analysed by two-way ANOVA followed by Bonferroni's post-test. Statistical significance was at $p<0.05$ (95% confidence interval) using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSIONS

The values of erythrogram are presented in the Fig 1. As described previously, only healthy animals with close values of erythrogram were included, consequently similar values resulted following AIP, in all three groups. The values in the 2nd day, before therapy, corresponded to a moderate anaemia (RBCs 7.48 ± 0.36 10^6/µL; HCT 21.99 ± 1.42 %; HGB 7.22 ± 0.45 g/dL), furthermore, following therapy, significant dif-

Table 1

Erythrocyte's indices in response to transfusion therapy; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cells deviation with (RDW)

No	MCV (fL)			MCH (pg)			MCHC (g/dL)			RDW (fL)		
	P	A	H	P	A	H	P	A	H	P	A	H
1	29.00±1.00	27.67±1.52	31.33±1.15	9.83±0.40	9.37±0.49	10.87±0.70	33.83±0.93	33.63±0.60	34.00±0.43	28.07±0.55	27.57±0.93	26.83±1.65
2	29.00±1.00	27.33±1.15	31.00±1.00	8.90±0.20	8.93±0.47	9.63±0.30	30.87±0.98	32.10±0.61	31.07±0.21	27.30±0.45	27.10±0.95	25.87±1.16
2	29.00±1.00	27.67±1.52	31.00±1.00	8.90±0.20	8.87±0.41	8.57±2.30	30.87±0.98	31.97±0.30	27.33±6.79	27.30±0.45	27.00±1.06	27.80±3.67
3	28.67±0.57	28.00±1.00	31.33±1.52	9.27±0.41	9.13±0.40	10.10±0.55	32.23±0.55	32.73±0.11	32.17±0.55	27.63±0.72	26.80±0.26	26.20±1.23
4	28.33±1.15	28.00±1.73	31.00±1.00	9.27±0.32	9.00±0.26	10.10±0.45	32.50±0.35	32.10±1.80	32.43±0.55	28.03±0.50	27.20±1.00	26.40±1.48
5	29.00±1.00	28.00±1.73	31.00±1.00	9.23±0.35	9.00±0.34	9.80±0.55	32.17±0.97	31.97±1.56	31.47±1.34	27.67±0.45	27.20±1.00	26.00±1.57
6	28.00±1.00	28.00±1.00	31.00±1.00	9.27±0.25	9.23±0.32	10.27±0.35	32.63±0.15	33.40±0.70	33.03±0.21	28.30±1.01	26.87±1.22	25.53±1.40
7	28.00±1.00	28.00±1.00	31.33±1.52	9.60±0.53	9.53±0.30	10.23±0.11	33.97±1.30	34.23±0.15	32.83±1.12	28.27±1.09	26.97±1.38	25.67±1.45
9	29.00±1.00	28.33±0.57	31.33±1.52	9.37±0.30	9.07±0.32	10.23±0.15	32.73±0.90	32.67±0.50	32.77±1.00	29.00±1.32	26.83±0.60	25.73±1.35
11	29.00±1.00	28.00±1.00	31.00±1.00	9.70±0.30	9.53±0.21	10.50±0.10	33.93±0.15	34.03±0.60	33.83±1.06	28.73±1.42	28.27±0.55	26.20±1.65
14	29.00±1.00	28.00±1.00	31.00±1.00	9.73±0.41	9.37±0.35	10.47±0.11	33.83±0.28	33.70±1.01	33.50±0.79	28.37±0.55	27.57±1.21	26.73±1.65
20	29.00±1.00	28.00±1.00	31.00±1.00	9.70±0.43	9.47±0.11	10.57±0.15	33.53±0.55	34.03±0.98	34.03±0.64	28.47±0.70	27.93±1.00	25.97±1.25

P – Control group, A – Autotransfusion group, H – Heterotransfusion group, (mean ± SD)
(n=3; two-way ANOVA followed by Bonferroni's post-test)

ferences were found. In response to fluid replacement therapy, immediately the animals showed slightly decreased parameters (RBCs 7.14 ± 0.08 10^6/μL; HCT 20.50±0.51%; HGB 7.27 ± 0.75 g/dL), they remained relatively constant up to the 7th day, when they started to rise, the trend maintained up to the 20th day when the surveillance ends. In the end of the study, the animals displayed only a mild anaemia (RBCs 9.12 ± 0.71 10^6/μL; HCT 26.42 ± 1.62 %; HGB 8.87 ± g/dL).

Contrariwise, the other two groups, receiving blood replacement therapy, exhibit a fast improvement; immediately after therapy the parameters were RBCs 8.67 ± 0.36 10^6/μL; HCT 25.18 ± 1.15 %; HGB 7.47 ± 0.32 g/dL for heterotransfusion group ($p< 0.05$), and RBCs 9.18 ± 0.59 10^6/μL; HCT ± %; HGB ± g/dL for autotransfusion group ($p<0.05$). At this stage no difference between those two groups was noticed. In the 3rd day, we noticed a slightly reduction in the heterotransfusion group but a significant difference was visible starting from the 4th day. In heterotransfusion group, the RBCs values remained lower than those of autotransfusion group did since the end of the study ($p<0.05$). They drop suddenly after the 4th day, but they remain below the values of control group ($p< 0.05$) until the day 14th. On the last two determinations, at the days 14th and 20th no differences between heterotransfusion group and control group were noticed. In autotransfusion group, similarly to heterotransfusion group, the therapy results in a rapid improvement of RBCs parameters, the difference became visible at the 3rd day, but it became more obvious in next days. A significant difference was maintained up to the end of the study ($p<0.05$). However, the values were not maintained constant up the beginning of the recovery stage. Best visible on the RBCs count, from the 5th day, the RBCs starting to decline, up to 7th day and remained constant up to day 14th. Elevated values were found only in the last determination on the day 20th. RBCs indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cells deviation with (RDW) shown small and irrelevant variations, remaining relatively constant during the entire study (Table 1). Similarly, the white blood cells and platelets showed no significant changes in response to transfusion therapy (data no shown).

In control group, the total proteins were slightly decreased in response to AIP, because of reduction of albumin fraction. Blood replacement therapy maintained them similar to initial values in both autotransfusion and heterotransfusion group (Table 2). No other important changes in plasma biochemistry were found. Direct and total bilirubin remained at similar levels, as well as the activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (data not shown).

In cattle, studies investigating the blood groups have a long history, starting to the 1940s; however, blood transfusion is seldom use in veterinary practice. The most important reason is the intricate system of blood groups (13). Cattle have 11 major blood group systems A, B, C, F, J, L, M, R, S, T, and Z, consisting in more than 70 antigens, the most of them included in B and C systems. The J antigen is not a true RBCs antigen it is plasma glycolipid, it is adsorbed on RBCs only when its plasma concentrations became sufficiently

Table 2

Variation of plasma proteins response to transfusion therapy

No	Total proteins (g/dL)						Globulins (g/dL)		
	P	A	H	P	A	H	P	A	H
1	6.37±0.31	6.63±0.51	6.17±0.50	3.03±0.15	2.83±0.29	2.90±0.26	3.33±0.21	3.80±0.79	3.27±0.29
2	5.83±0.25	5.90±0.61	5.77±0.15	2.83±0.12	2.57±0.25	2.87±0.21	3.00±0.17	3.33±0.84	2.90±0.17
2	5.83±0.25	5.60±0.26	5.73±0.06	2.83±0.12	2.73±0.06	2.80±0.10	3.00±0.17	2.87±0.32	2.93±0.12
3	5.53±0.12	6.13±0.32	6.03±0.15*	2.60±0.10	2.67±0.32	2.83±0.06*	2.93±0.15	3.47±0.64	3.20±0.10
4	5.63±0.15	6.23±0.78	6.27±0.25*	2.67±0.12	2.70±0.17	2.90±0.10*	2.97±0.25	3.53±0.95	3.37±0.15
5	5.63±0.15	6.27±0.31	6.10±0.44	2.60±0.10	2.57±0.25	2.80±0.10	3.10±0.20	3.70±0.56	3.30±0.35
6	5.70±0.17	7.00±0.70	6.73±0.95	2.57±0.12	2.57±0.23	2.93±0.23	3.57±0.15	4.43±0.91	3.80±0.75
7	6.13±0.06	6.90±0.36	6.57±0.58	2.83±0.12	2.77±0.23	2.93±0.06	3.67±0.49	4.13±0.59	3.63±0.55
9	6.50±0.44	7.17±0.12	6.73±0.40	2.77±0.15	2.77±0.25	2.87±0.12	3.97±0.15	4.40±0.36	3.87±0.32
11	6.73±0.15	7.23±0.76	7.23±0.80	2.83±0.15	2.70±0.17	2.93±0.15	4.50±0.44	4.53±0.93	4.30±0.75
14	7.67±0.32	7.50±0.17	7.47±0.76	2.83±0.15	2.70±0.17	2.90±0.10	4.83±0.47	4.73±0.32	4.57±0.80

P – Control group, A – Autotransfusion group, H – Heterotransfusion group, (mean ± SD)
(n=3; two-way ANOVA followed by Bonferroni's post-test), (* p<0.05, as compared to Control group)

high. J negative cattle have anti-J antibody, and develop haemolysis if transfused with J positive blood (3).

Expectedly, in our study, the efficiency of heterotransfusion was very limited, the RBCs parameters started to decline only two days following transfusion, and three days later, they were similar to those of the control group. However, blood transfusion seems to maintain the albumin levels, and haemolysis did not influence other parameters as bilirubin levels.

On the other hand, autotransfusion was less efficient than expected either. As described above, RBCs removal occurs in autotransfusion group from day 5th to day 7th, however the process was not as destructive as in case of heterotransfusion group; in autotransfusion group the values of erythrogram were always higher than those of control and heterotransfusion groups.

The reason why RBCs life span was reduced by autotransfusion may only be presumed at this stage. It might be caused by preservation at 4ºC, over nigh, or mechanical stress to which the RBCs were subjected during transfusional procedures, but regardless of the reason those finding suggests that the efficiency of the blood transfusion might be improved by refinement of transfusional procedures.

However, we noticed the distinct improvement in RBCs levels, throughout the entire study, in patients subjected to autotransfusion as compared to those receiving heterotransfusion; showing that the RBCs removal was mediated mainly by immune response, other mechanisms being marginal.

CONCLUSIONS

Blood transfusion in cattle had very low efficiency, the RBCs parameters started to decline only two days following transfusion, and three days later, they were similar to those of the anaemic group no receiving transfusion. In the next days, blood transfusion had no influence on the recovery; RBCs displayed a similar dynamic in transfusion group and anaemia non-treated group.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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